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EDITORS
CHARLES A. SHULL
CHARLES B. LIPMAN
CARLETON R. BALL
BURTON E. LIVINGSTON
FRANCIS E. LLOYD

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ERRATA

Page 90, first line of citation no. 26, for "or" read of.
Page 154, third line of citation no. 24, for "nedelandsch" read Nederlandsch.
Page 184, line 16, for "confined" read confirmed.
Page 200, first column of table III, fourth line, for "A. M. 21" read A. M. 22.
Page 427, line 8, for "decreases" read decrease.

JULIUS VON SACHS
1832–1897

To

JULIUS VON SACHS

BORN OCTOBER 2, 1832

THIS VOLUME OF PLANT PHYSIOLOGY IS DEDICATED

IN HONOR OF

THE ONE-HUNDREDTH ANNIVERSARY OF HIS BIRTH

PLANT PHYSIOLOGY

JANUARY, 1932

WILTING AND WITHERING OF GRASSES IN GREENHOUSE CULTURES AS RELATED TO WATER-SUPPLYING POWER OF THE SOIL[1]

J. DEAN WILSON AND BURTON E. LIVINGSTON

(WITH FOUR FIGURES)

Introduction

It has recently been emphasized (**14, 15, 19, 20, 32**) that the two main environmental conditions that influence the development and health of ordinary leafy plants, as far as water relations are concerned, are (a) the capacity of the soil to deliver water to the plant roots and (b) the capacity of the aerial surroundings to permit water loss from the foliage through transpiration. The second of these conditions may be measured and recorded in a fairly satisfactory way, by means of atmometers of various forms, and numerous studies on the relations between evaporation and plant health have been reported in the literature of plant physiology and ecology. While very much more remains to be learned about the influence of this complex climatic feature on plant activity, it is safe to say that our knowledge of evaporation relations and our methods for securing instrumental records of fluctuations in the intensity of evaporation conditions are now far more advanced than are our knowledge and methods with regard to the influences exerted on plants by soil-moisture conditions. What happens to an ordinary plant in the region below the soil surface still remains a subject for rather free conjecture. Soil science furnishes much that is relatively definite, consistent and apparently reliable in its field, but the points of contact between our knowledge of plant physiology and our knowledge of soil physics and soil chemistry are still comparatively few and are generally unsatisfactory. The soil system is so complex that it has not readily lent itself to the usual analytical methods and our present great fund of information about soil moisture leaves us still unable to integrate the many separate portions of that information in any way that would permit clear insight into the water relations of root systems. It appears that the analytical methods that have yielded valuable principles and generalizations in the

[1] Botanical contribution from the Johns Hopkins University, no. 92.

fields of physics and chemistry are not as yet very promising when we aim to study the relations between two such very complex systems as the plant and the soil. To further such a study it is essential that primary attention be given to the soil system considered as a whole, avoiding the distractions of detail that confound us along the paths of the more thoroughly analytical method as generally employed.

Roots are, of course, unable to absorb any water that has not already somehow come into contact with their absorbing surfaces, and the maximum rate at which a given root system may absorb water can not surpass the rate at which root surfaces and soil moisture come into contact. If for the moment we consider the absorbing surfaces of the root system as fixed in position and extent, then the maximum rate of absorption is limited at any instant by the rate at which the soil system can supply water to the root-soil surfaces. Conditions within the plant should control the rate of absorption as long as the latter is slower than the highest possible rate of external supply, but soil conditions should control the absorption rate whenever the soil system is unable to bring water to the absorbing surfaces as rapidly as it would be absorbed if the rate of supply were more rapid. Consequently the primary soil condition that may limit the rate of entry of water into stationary roots is the rate at which the soil system can deliver water to these organs. This has been called the water-supplying power of the soil (**2, 5, 6, 11, 12, 16, 17, 18,** [p. 115], **22, 32**), that is, its capacity as a mechanism, to deliver water at a unit of water-absorbing surface.

This dynamic characteristic of the soil around the roots of a plant is, of course, the resultant of secondary, contributory or component soil characteristics, such as the kind of soil (measured as its water-holding power, its moisture equivalent, etc.) and its state of packing, its water content per unit of natural volume, and its temperature. These are static characteristics that may be more or less satisfactorily measured from time to time, but measurements of them are of very slight value in physiology, ecology, agronomy, horticulture, sylviculture, etc., because it is as yet impossible to deduce from such measurements a numerical index of water-supplying power for any time period. Such an index should differ in magnitude with the kind of soil, with its degree of packing, with its moisture content, with its temperature, and perhaps with other static features. Its indirect derivation, for a given soil region, from the results of measurements of these components taken separately must consequently remain complex and tedious in the extreme.

Direct measurement of the water-supplying power of the soil near the roots of the plant appears to offer a promising means by which we may hope to improve an understanding of the dynamic water relations between plant and soil. The only feasible and tested method thus far available for securing direct measurements of this dynamic soil feature is the soil-point method first brought forward by LIVINGSTON and KOKETSU (**17**). A suitable artificial absorbing surface, of porous porcelain (or wood), is applied to the soil at the depth considered and the capacity of the soil to supply water is measured as the amount of water actually absorbed in a unit of time. The water-supplying power is therefore the rate at which the given soil, at the given depth, is capable of delivering water to a stationary absorbing surface. This is a dynamic variable, the magnitude of which fluctuates. It does not directly influence the actual rate of water absorption by a root unless the absorbing capacity of the root surface exceeds it in magnitude. when it acts to limit the rate of absorption. Direct soil-point measurements of the water-supplying power of the soil may be made at suitable intervals, at any required depths in the soil, and graphs may be constructed from them to show fluctuations in water-supplying power throughout a period of time. The operations are relatively very simple and rapid (though the method is surely susceptible of further improvement) and no intricate calculations are required. The soil mechanism itself is allowed to integrate the several component terms or factors or arguments (in the sense of the mathematical calculus) and the data secured are measurements of the resultant only. The observer measures directly the function of several variables instead of measuring the variables and then attempting to calculate the value of the function from the results of his measurements.

The soil points used in this study were like those employed by WILSON (**32**). The instrument is a hollow porous-porcelain piece with wall about 3 mm. thick. It has a cylindrical part open at the top and continuous below with a conical part that ends in a sharp point. The cylindrical part is 2.4 cm. in outside diameter and 2.5 cm. high. The outer surface is thoroughly waterproofed near the point, also throughout the cylindrical part and the upper portion of the conical part, and a band 2 cm. high on the conical part is left uncoated. This zone has a diameter of about 2.2 cm. at its upper margin. It presents to the soil into which the instrument is thrust an absorbing surface of about 12 sq. cm.

It has been mentioned or implied by PULLING and LIVINGSTON (**25**), PULLING (**24**), WILSON (**32**), and VEIHMEYER (**27, 28**) that the initial water-supplying power of the soil adjacent to a water-absorbing surface (such as that of plant root or artificial absorber) is not maintained in the case of aerated soils with water content below maximum field capacity unless nearly saturated soil is closely adjacent to the soil region considered. When an absorbing surface with sufficient capacity for water absorption is brought into capillary contact with moist soil the rate of water delivery from the soil is initially at a maximum and rapidly decreases. The water nearest to the absorber is taken up promptly but the rate of subsequent absorption is limited by the rate at which water from more distant soil films migrates to the absorber. This rate of migration becomes very slow, as has been emphasized by VEIHMEYER, when the additional water must move a considerable distance before being absorbed, and the water-supplying power of the soil therefore decreases rapidly as absorption proceeds from such soils. It may happen in some cases that water distillation from soil films to root surface is of some importance in maintaining health with low transpiration rates, but it seems improbable that plant health would generally be long maintained without movement of liquid water from soil to root.

There seems to be no reason to doubt that roots in moist soils experience this progressive decrease in the dynamic availability of water adjacent to their absorbing surfaces. When an absorbing surface of a root initially comes into contact with a new system of soil films, by the penetration of root tip or root-hair into untouched soil, absorption may be relatively rapid and the water-supplying power of the soil system may be greater for a time than the absorbing power of the root surface. If absorption continues, however, supposing the absorbing power to remain the same as at first, there eventually comes a time when the supplying power of the soil is smaller than the absorbing power of the root surface, and the supplying power of the soil then becomes the limiting condition for the process of water absorption. It follows, as has been recently emphasized by VEIHMEYER, WILSON and others, that a root system in a soil that is merely moist and does not become wetter can generally neither maintain nor increase an already considerable rate of water absorption unless the roots continue to advance into new soil at an adequate rate.

It is probable that one of the effects of the onset of inadequacy in the water-supplying power of the soil about the roots of a plant is generally a retardation leading toward the ultimate cessation of root advance, for root growth and the formation of root-hairs requires additional water, and water that has been fixed by growth may be considered as temporarily held in the turgid tissues, being therefore generally removed from consideration. If water can move at an adequate rate into the growing region of root or root-hair, from the older regions (perhaps ultimately from some water-storage tissue of the plant or from the absorbing surfaces of another portion of the root system that may still absorb water more rapidly than it is conducted to other parts), then it should be possible for some root and root-hair growth to continue after the water-supplying power of the adjacent soil has become too low to support root growth.[2] It may even sometimes happen that a root may elongate at the expense of water of respiration (**1**), the root surfaces continuing to advance slowly into new soil regions where the water-supplying power is already inadequate. (Of course water of respiration does not enter the root at all; it is chemically formed in the tissues through the oxidation of organic material that has migrated hither from some other part of the plant.) But it appears highly probable that inadequacy in the capillary water-supplying power of the soil adjacent to the absorbing portions of a root system generally soon makes itself effective on the plant by retarding or stopping root enlargement (**8**). If this represents a general truth, then the continuous growth of roots is of basic importance for the maintenance of the rate of water absorption from soils that are characterized by relatively low water-supplying power, a consideration that does not thus far appear to have been seriously considered in the discussion of soil moisture conditions and water absorption by plants.

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[2] HENDRICKSON and VEIHMEYER (8) report that this sort of root growth was not observed in experiments planned to throw light on some of the possibilities here mentioned.

WEAVER (**30**, p. 44), in discussing root growth in field crops, mentions that root-hair growth makes fresh stores of water and solutes available to the plant and calls attention to the fact that but few root-hairs are produced by field crops in wet soils, while, if the plants do not wilt meanwhile, the greatest development of root-hairs may occur with soil-moisture contents somewhat less than would give the highest crop yields. It may be that some plants with slightly inadequate water supply (in their apparent "endeavor" to reach new sources of water) may produce root-hairs and small roots at the expense of the rest of the plant, with respect to water as well as with respect to other materials. This may act to hinder crop production and may thus limit the final yield to some extent.

The importance of the actual process of root enlargement is probably very great for upland plants, especially in times of more or less pronounced drouth. At such times the water-supplying power of the soil is relatively low and the wiping-up action of advancing roots and root-hairs should be very essential to the continued development of the plant. Such a plant can not long maintain its vigor and at the same time wait for the soil system to deliver water to its absorbing surfaces; these surfaces must be extended rapidly into less depleted regions of the soil, or else growth must soon become greatly retarded or cease entirely. It consequently follows that the instrumentally determined indices of water-supplying power that are requisite for the dynamic study of plant water relations should represent the *initial* water-supplying power for each test; that is, they should represent the condition encountered by a growing root or root-hair when it *first* comes into the soil region considered.

The soil-point method is especially suited to the determination of these initial values. As recently employed, this method gives values of the supplying-power index that represent only the first hour after an absorbing surface is brought into capillary contact with the soil surface tested. Consecutive tests may, of course, be made by applying several instruments, one after another to the same soil surface, and valuable information may thus be secured regarding the rate of decrease in the water-supplying power as absorption proceeds.

If a soil is very wet, water absorption may continue for a long time after the absorbing surface of a growing root comes into contact with any particular portion of soil. With drier soils this period of absorption is limited, as has been said, and if the soil is sufficiently dry no absorption may occur. The period during which absorption may occur is shorter the drier is the soil into which the absorbing surfaces are thrust, and some specific degree of dryness should correspond to the single-hour period employed in connection with the soil-point method. If a soil region is so dry that newly formed roots find its water-supplying power inadequate to their needs within an hour after they have made contact with the delivering surface, it is safe to say that such a soil region generally has a water-supplying power too low for successful development of the plant. Periods much shorter than one hour are inconvenient in the application of the soil-point method and periods much longer introduce more and more the danger that the porous porcelain of the instrument may become so wet within the period that its own absorbing power becomes the thing measured, rather than the water-supplying power of the soil. This undoubtedly occurs with very wet soils, but it probably does not generally occur with supplying powers of less than 1000 mg. per soil point for the single hour. As WILSON (**32**), MARSHALL (**21**) and others have shown, the one-hour period is well suited to the study of soil water-supplying powers in relation to plant health.

Before bringing this introduction to a close it is essential to mention again a point emphasized by WILSON, that the insertion of a soil point into the soil inevitably involves some alteration in the water-supplying power at the very surface where the test is to be made. The soil is compressed laterally and to some extent downward as the conical opening is made for the insertion of the instrument. Therefore, the soil adjacent to the instrument is generally somewhat different in water-supplying power from the unmodified soil.' With progressively lower values of the supplying-power the effect of this artificial compression becomes less, however, and the artifact thus introduced may safely be considered as very small when the index values dealt with approach the critical magnitudes about which growth ceases, wilting begins, etc. In any event, the effect of artificial compression is always relatively small and appears to be quite negligible in comparison with the differences in water-supplying power observed for different places at the same depth in the same soil mass. When greater precision becomes desirable in the measurement of water-supplying power the method may perhaps be modified so as to avoid or at least greatly diminish this error.

The present paper reports the results of a study of the progress of the wilting, withering and death of originally healthy and vigorous greenhouse plants in winter, after watering had been discontinued. The study dealt with the progressive decrease in the water-supplying power of the soil about the roots as the soil dried out. The experimentation was carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, in the fall and winter of 1925–26.

Most of the cultures used were in shallow trays or flats but a few tests were made with pot cultures. The three greenhouse rooms of the Laboratory of Plant Physiology were used, several cultures being placed in each room. The soils of the different flats and pots were all approximately alike. The climatic conditions were about the same for all the cultures in each greenhouse room and they were not very greatly different for the different rooms. The cultures in flats will be considered first in the following pages and a brief section will be devoted to the results secured with pot cultures.

Cultures in flats

METHODS

Seventeen different grasses were planted in flats, two or more species in each flat. Two clovers commonly found in lawns were also added to the group. Each kind of plant occupied an area about 80 x 70 cm., and a similar area without plants served as a control for each greenhouse room. Each flat was filled with soil to a depth of about 18 cm., little attention being given to packing since these cultures were to be watered in the ordinary way for a period of several months before critical observations were to be begun. The soil used was a friable mixture, such as that commonly used in these greenhouses, made up of one part each of sand and leaf-mold and three parts of a rather heavy loam (Sassafras loam). The water-holding capacity of the mixture was, by the HILGARD method, 44 per cent. on the basis of dry volume, or 50 per cent. on the basis of dry weight. Enough soil was prepared to care for these cultures and also for the pot cultures that were to be started subsequently.

The flats were planted early in June, 1925, either by sowing seeds or planting stolons, and the plants were allowed to grow, with watering as needed, until December 30, when they were uniformly in excellent condition. The flats were all thoroughly watered by sprinkling on the last-named date and the two spheres of a radio-atmometer (**13**), one white and one lampblack-coated, were installed at corners of one of the flats in each greenhouse room, each sphere being about 25 cm. above the soil surface. In the second (middle) room a Richard thermograph was placed in a shaded but well ventilated position, to record temperature fluctuations, which were practically the same in all three rooms.

On December 31, soil-point readings on these cultures were begun, for the 6-cm. depth, and these were continued at intervals of three days until February 14. The soil points used in these tests, which had been carefully selected for uniformity in weight and absorptive capacity, were dried in the air for two days after each determination and then placed in a desiccator until needed for use again. Two points were used for each observation and the reading was obtained by dividing by 2 the total gain in weight for the usual one-hour period of exposure in the soil. To prevent increased evaporation from the soil and other possible disturbances, the little holes left on removing the soil points were always immediately filled with soil from the reserve supply. At each time of observation a test was made for the soil areas without plants. The different plant areas in any flat were not considered separately until some of the plants began to show slight effects of drouth, on January 18. Throughout this preliminary period, while all the plants were still vigorous, a soil-point reading was secured for each of the ten flats at each time of observation. On January 18 and at later times of observation readings for all plant areas, as well as for the control area, were made at about the same time. These observations were continued at 3-day intervals, as has been said, throughout the period while the plants passed through various successive stages of drouth injury and until the leaves of all but two of the forms were dead, the two exceptions (red fescue and sheep's fescue) being very drouth resistant.

On February 14 the cultures were all again thoroughly watered and then kept well supplied with water throughout the following month, to ascertain whether the plants in each case would recover when an adequate water supply was again maintained. This treatment resulted in recovery such that red fescue, sheep's fescue, Kentucky blue-grass and Canadian blue-grass were in good condition on March 14. With awnless brome-grass, tall oat-grass and orchard grass there was no recovery, while all of the other forms tested had returned to fair condition by the end of this second period of watering.

The atmometers were read every three days, at each time when soil-point readings were taken, and the average daily, corrected water losses for the 3-day period, from the white and from the blackened sphere, were recorded for each room of the greenhouse. Corresponding averages for the mean temperatures for each 3-day period were secured from the thermograph tracings for the middle room. Also, the growth conditions of the corresponding plants were recorded when soil-point readings were made. Numerical values or scores were given, referring to five different degrees of health, based on the apparent condition of the leaves, as follows: 4, plants in excellent condition; 3, condition good but not excellent; 2, condition poor, with leaves wilting; 1, badly wilted but partly recovered after 18 hours in a moist chamber; 0, badly wilted, with no recovery after 18 hours in a moist chamber, many of the leaves being gray and crisp. It was noted that these grass leaves, which wilted and withered in the greenhouse in the winter season, generally failed to develop the brown colors that are so characteristic of most of these forms when their leaves die from the effects of drouth in the open at Baltimore. The dead leaves of these greenhouse-grown grasses became greenish or silvery gray upon drying-up and remained so for some time afterwards. They became brown, however, after several weeks of subsequent exposure in the greenhouse. This difference may be due to difference in light effects, the summer light acting to alter the color of the leaves more rapidly.

An attempt was made to detect the beginning of a rather definite stage of bad wilting, similar to the stage called the beginning of "permanent wilting" by BRIGGS and SHANTZ (**3**). It will be remembered that permanent wilting is defined as a degree of wilting from which the plant fails to recover when its transpiration is practically stopped, but from which it recovers (although slowly in many cases) if given adequate water supply about its roots. BRIGGS and SHANTZ exposed their supposedly permanently wilted plants in a saturated atmosphere for an undefined period and recorded them as permanently wilted only when they failed to show recovery. CALDWELL (**4**) and SHIVE and LIVINGSTON (**26**) employed a 24-hour period for this moist-chamber test. In the present studies the test was applied for 18 hours (including the night period) instead of 24, by inverting over a group of the plants in question a glass crystallizing dish about 15 cm. in diameter and about 8 cm. in depth. The edge of the inverted dish was pressed firmly against the soil and the enclosed air-chamber must very soon have become practically saturated with water vapor. These tests thus consisted in surrounding the leaves with nearly water-saturated air for a period of approximately 18 hours, during which time there was no significant transpiration.

In most cases where the moist-chamber test just described was applied no recovery was noted at the end of the test period and it was considered that the plants that failed to recover had at least passed beyond the particular stage of wilting here used as approximately corresponding to the beginning of permanent wilting as defined by BRIGGS and SHANTZ. These plants were given the score value of 0, being classed as vegetatively dead. In some other cases considerable recovery (of about half or more of the leaves) was manifest at the end of the 18-hour period under the crystallizing dish and the plants showing such recovery were given the numerical score value of 1, very poor.

This method of determining the soil conditions coincident with the attainment of a particular stage of wilting might easily be made considerably more precise, especially if the soil-point readings were more frequently taken. However, as the plants approached the critical stage of wilting here considered, the rate of decrease in the water-supplying power of the soil was very slow. It is consequently probably safe to say that the plants given the growth-index value of 0 had not passed the critical wilting point by more than three days at most, and that no plants given the index value of 1 had yet reached that stage of wilting.

PLANTS USED

Two species of clover and seventeen different grasses, the latter including nine different genera, were used. Some of these are common on lawns of the Baltimore region and elsewhere, while others are often used in agricultural practice. They are known to differ widely in general drouth resistance on lawns and in meadows and they consequently offer opportunity for making selections to suit different climates and soils. These nineteen forms were as follows: (**9, 23**) : Alsike clover (*Trifolium hybridum* L.), white clover (*Trifolium repens* L.), Kentucky blue grass (*Poa pratensis* L.), Canada blue grass (*Poa compressa* L.), red fescue (*Festuca rubra* L.), sheep's fescue (*Festuca ovina* L.), Chewing's fescue (*Festuca* sp.), Rhode Island bent grass (*Agrostis tenuis* Sibth.), velvet bent grass (*Agrostis canina* L.), Vermont bent grass (*Agrostis* sp.), Washington bent grass (*Agrostis* sp.), perennial rye-grass (*Lolium perenne* L.), Bermuda grass (*Capriola dactylon* Adans.), tall oat-grass (*Arrhenatherum elatius* L.) brome grass (*Bromus inermis* Leyss.), orchard grass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), redtop (*Agrostis palustris* Huds.), meadow fescue (*Festuca elatior* L.).

NUMERICAL DATA

Our temperature and evaporation data are presented in table I and the corresponding soil-moisture and plant data are given in table II. The dates of observations are indicated across the top of the table in each case. The temperature values are given in degrees Centigrade, each value representing the average of the three daily means for the 3-day period indicated. The mean for each day was the average of the maximum and minimum for the day, as these were shown on the thermograph tracing, and the three daily means for each period were simply averaged to represent the 3-day period. Each evaporation value represents the average daily, corrected water loss (in cubic centimeters) from the blackened atmometer sphere (*E*) or from

TABLE I

Mean daily temperatures and corrected evaporation values for greenhouse cultures, for 3-day periods, from Dec. 31, 1925, to Feb. 14, 1926

3-DAY PERIOD ENDING	JAN. 3	JAN. 6	JAN. 9	JAN. 12	JAN. 15	JAN. 18	JAN. 21	JAN. 24	JAN. 27	JAN. 30	FEB. 2	FEB. 5	FEB. 8	FEB. 11	FEB. 14
Temperature (*Deg. C.*)															
Room II of greenhouse	18	19	15	16	15	16	21	15	16	16	18	17	17	17	19
Evaporation (*cc.*)															
Room I of greenhouse:															
Blackened sphere (E)	22.7	12.7	17.7	18.7	20.0	16.3	22.7	20.0	23.3	21.3	15.0	20.7	20.3	18.0	20.0
White sphere (e)	18.3	11.3	14.7	15.7	18.0	15.3	19.3	17.3	20.0	18.0	13.3	17.7	17.7	15.3	16.7
Sunshine effect ($E-e$)	4.3	1.3	3.0	3.0	2.0	1.0	3.3	2.7	3.3	3.3	1.7	3.0	2.7	2.7	3.3
Room II of greenhouse:															
Blackened sphere (E)	19.0	11.7	15.3	16.3	18.3	15.3	19.7	16.3	19.7	17.7	12.7	17.7	19.0	14.0	17.0
White sphere (e)	17.3	10.7	13.7	14.7	16.7	14.0	17.7	14.7	17.3	16.0	12.0	16.3	17.7	13.0	15.7
Sunshine effect ($E-e$)	1.7	1.0	1.7	1.7	1.7	1.3	2.0	1.7	2.3	1.7	0.7	1.3	1.3	1.0	1.3
Room III of greenhouse:															
Blackened sphere (E)	21.0	11.7	17.0	18.0	20.3	16.7	21.3	19.7	22.7	19.7	14.3	18.7	20.0	16.7	20.0
White sphere (e)	18.0	10.0	14.3	15.0	17.3	15.3	19.0	16.3	19.0	17.3	13.0	16.3	17.3	15.0	17.3
Sunshine effect ($E-e$)	3.0	1.7	2.7	3.0	3.0	1.3	2.3	3.3	3.7	2.3	1.3	2.3	2.7	1.7	2.7

the white sphere (*e*), for the 3-day period. It was secured by dividing by 3 the corrected total loss for the 3 days in each case. Each actual loss observed was, of course, first multiplied by the coefficient of the atmometer sphere from which the loss had occurred. The value for the sunshine effect (*E–e*) for each 3-day period is shown (in cubic centimeters) as the difference between the mean daily corrected loss from the blackened sphere and the corresponding mean daily loss from the white sphere. These atmometer data are arranged in three groups, for the first, second and third rooms of the greenhouse.

The data for the initial water-supplying power of the soil (table II) are expressed in terms of milligrams of water absorbed per soil point, for the 1-hour period and for the 6-cm. depth. Each index value represents the number of milligrams supplied to the soil point, across a surface of about 12 sq. cm. Thus an index value of 100 indicates that the soil could supply water to the absorbing surface at a rate of about 8 mg. per square centimeter, for the first hour. The different plant forms considered are named at the left of the table and the room of the greenhouse in which each form was grown is indicated at the extreme right. The plant condition, as this became poorer with the drying-out of the soil, is shown for each time of observation, as follows: The soil values for condition 4 (vigorous growth) are in Roman type, those for condition 3 (some retardation) are in Italics, and those for condition 2 (beginning of definite injury), for condition 1 (very poor) and for condition 0 (vegetatively dead) are all in bold-face type. The values for condition 1 and 0 are distinguished from each other and from those for condition 2 by the introduction of a capital letter before each value, P for very poor (condition 1) and D for dead (condition 0). All values for plants in the vegetatively dead condition are separated from the rest of the table by a stepped line.

The soil area without plants (control) is represented in table II by the first line and the plant names are arranged in the order of the dates of onset of vegetative death, those at the bottom of the list representing plants that retained bright green and apparently healthy leaves longest after watering of the cultures had ceased. When the onset of vegetative death is recorded for several plant forms on the same day the plant names are arranged according to the decreasing order of magnitude of the corresponding soil-moisture indices. Tall oat-grass and orchard grass are recorded as having all their leaves dead on January 27, while Canada blue-grass did not reach that stage of withering till February 14 and sheep's fescue and red fescue did not reach it at all in the period of the experiment. The other forms take intermediate positions in the list.

It is apparent that there is a general tendency for the soil-moisture indices corresponding to the beginning of vegetative death to be larger in

TABLE II

INITIAL WATER-SUPPLYING POWER OF THE SOIL AND PLANT CONDITION AT END OF EACH 3-DAY PERIOD, DEC. 31, 1925, TO FEB. 14, 1926

DATA ARE EXPRESSED AS MILLIGRAMS OF WATER ABSORBED PER SOIL POINT, FOR THE 1-HOUR PERIOD AND FOR THE 6-CM. DEPTH

VALUES IN ROMAN TYPE CORRESPOND TO VIGOROUS PLANT CONDITION, THOSE IN ITALICS CORRESPOND TO PLANTS SOMEWHAT RETARDED IN GROWTH, WHILE INJURY TO THE PLANTS IS INDICATED BY BOLD-FACE TYPE

MARKED INJURY IS SHOWN BY THE PREFIXED LETTERS, P (VERY POOR CONDITION) AND D (VEGETATIVELY DEAD)

PLANT FORM	DEC. 31	JAN. 3	JAN. 6	JAN. 9	JAN. 12	JAN. 15	JAN. 18	JAN. 21	JAN. 24	JAN. 27	JAN. 30	FEB. 2	FEB. 5	FEB. 8	FEB. 11	FEB. 14	ROOM OF GREENHOUSE
Controls (no plants)	3003	2967	2784	2123	1396	749	486	259	135	75	56	·49	40	42	34	28	
Tall oat-grass	3275	3005	2717	2040	*1365*	*475*	*261*	**91**	**P58**	**D52**	**D45**	**D40**	**D39**	**D49**	**D36**	**D28**	I
Orchard grass	3095	2991	2864	2240	*1219*	*630*	*197*	**82**	**P57**	**D42**	**D40**	**D42**	**D43**	**D43**	**D36**	**D33**	I
Brome grass	3095	2991	2864	2240	*1219*	*630*	*251*	**94**	**60**	**P49**	**D42**	**D38**	**D29**	**D39**	**D32**	**D26**	I
Rhode Island bent grass	2940	2729	2557	1875	1251	*963*	*360*	*142*	**80**	**P51**	**D40**	**D40**	**D37**	**D37**	**D32**	**D28**	I
Velvet bent grass	3140	3008	2922	2810	*2011*	*1110*	*908*	*225*	**106**	**P86**	**P48**	**D44**	**D37**	**D34**	**D31**	**D42**	I
Meadow fescue	2722	1889	1414	850	*243*	*133*	*93*	*75*	**60**	**65**	**P49**	**D43**	**D38**	**D42**	**D34**	**D26**	II
White clover	3003	2852	2540	1967	*934*	*453*	*157*	100	**59**	**P47**	**P44**	**D42**	**D38**	**D36**	**D33**	**D18**	III
Vermont bent grass	2722	1889	1414	850	*243*	*133*	*86*	*69*	**55**	**54**	**P42**	**P49**	**D40**	**D37**	**D35**	**D38**	II
Timothy	3245	3045	2890	2304	1896	*1097*	*579*	*360*	**99**	**80**	**50**	**P49**	**D37**	**D41**	**D36**	**D30**	I
Washington bent grass	3140	3008	2922	2810	2011	1110	*707*	*213*	**61**	**55**	**P48**	**P49**	**D36**	**D32**	**D38**	**D28**	I
Bermuda	2940	2729	2557	1875	1251	*963*	*466*	*265*	**101**	**73**	**58**	**P48**	**D34**	**D34**	**D36**	**D29**	I
Chewing's fescue	3166	2977	2590	1296	567	*299*	*223*	*117*	**58**	**48**	**P39**	**P39**	**D32**	**D34**	**D32**	**D39**	I
Alsike clover	3003	2852	2540	1967	*934*	*453*	*176*	*135*	**85**	**59**	**P51**	**P35**	**D30**	**D36**	**D33**	**D29**	III
Redtop	3245	3045	2890	2304	1896	*1097*	*656*	*325*	*81*	**56**	**52**	**52**	**P41**	**D37**	**D32**	**D28**	I
Kentucky blue grass	3022	2653	2222	1398	707	*495*	*395*	*329*	*165*	**87**	**73**	**62**	**P45**	**P41**	**D41**	**D23**	II
Perennial rye grass	3022	2653	2222	1398	*707*	*495*	*325*	*225*	100	**66**	**55**	**P55**	**P48**	**P48**	**D38**	**D35**	II
Canada blue grass	3022	2653	2222	1398	707	495	*399*	*293*	*133*	**81**	**75**	**72**	**51**	**P50**	**P41**	**D35**	II
Sheep's fescue	3275	3005	2717	2040	1365	*475*	*291*	*123*	*60*	**48**	**36**	**P40**	**P33**	**P31**	**P29**	**P27**	I
Red fescue	2722	1889	1414	850	243	133	*99*	*85*	*64*	**52**	**47**	**41**	**32**	**P33**	**P35**	**P29**	II

the upper part of the table and smaller in the lower part. Tall oat-grass showed all its leaves dead with a water-supplying power of 52, while red fescue and sheep's fescue had not reached that stage with supplying powers of 29 and 27 respectively. If the plant names are arranged according to the descending order of their soil-moisture indices when vegetative death was first noted, the list takes the form shown below, which is somewhat different from the list of table II, the index value being shown opposite the plant name in each case.

Tall oat-grass	52	Timothy	37
Velvet bent grass	44	Redtop	
Meadow fescue	43	Washington bent grass	36
Orchard grass	42	Canada blue grass	35
Brome grass		Bermuda grass	34
White clover		Chewing's fescue	32
Kentucky blue grass	41	Alsike clover	30
Rhode Island bent grass	40	Red fescue	29
Vermont bent grass		Sheep's fescue	27
Perennial rye-grass	38		

Tall oat-grass is again shown as least resistant while sheep's fescue and red fescue are shown as most resistant. The difference between the values for adjacent forms in the intermediate region are, in many cases, probably not significant. It is notable that tall oat-grass stands by itself, having a much higher index value (52) than the next form on the list, velvet bent grass (44). The upper fourth of the total range of values (27–52) is represented by tall oat-grass alone. The lower fourth is represented by Chewing's fescue, alsike clover, red fescue and sheep's fescue.

Graphs

The data for the twenty soil areas were plotted in graphic form for convenient study and four of the resulting graphs (for the control area without plants and for the soils with tall oat-grass, with Kentucky blue-grass and with red fescue) are shown in figures 1–4, these being representative samples. The values of the water-supplying power of the soil for the several dates of observation form the ordinates. The abscissa for any ordinate represents the total accumulated evaporational water loss from the corresponding blackened atmometer sphere for the period beginning December 31 and ending at the time of the observation in question. Each graph thus represents primarily the rate of decrease in the soil-moisture value as related to the corresponding evaporation conditions and as related to the lapse of time. All four graphs are on the same scale. Dates of observation are shown above and the evaporation values below. The scale for soil moisture values is shown at the left. On the three plant graphs the health condition of the plants is shown by the vertical subdivision of the area of the graph, each part bearing a notation that is self explanatory. The broken horizontal line near the base of each graph indicates the ordinate corresponding to the soil-moisture index value of 100, which has been emphasized as critical by WILSON (**32**).

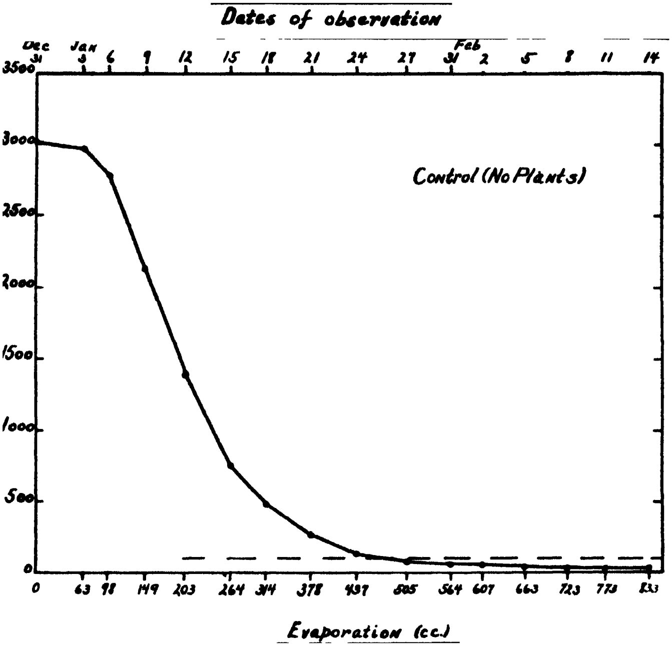

FIG. 1. Graph showing decrease in water-supplying power (ordinates) for 6-cm. depth in control soil mass, without plants, as related to lapse of time (abscissas, above) and cumulative evaporation from standard blackened atmometer sphere (abscissas, below), as soil dried out after final watering. Horizontal broken line represents supplying-power value of 100.

The average water-supplying power of the soil at the time of the first observation on December 31, twenty-four hours after the soil in the flats had been saturated with water, was in all cases about 3,000. This is markedly greater than the average value generally secured in WILSON's (**32**) studies of a lawn soil out-of-doors after a hard rain, which is only about 2,000. Although the greenhouse flats were well drained, the height of their soil columns was very short in comparison with that for the open lawn, and the moisture content at the 6-cm. depth when drainage had just ceased was consequently much higher in the flats.

From this high value at the start the index of water-supplying power decreased as the soil dried out, the beginning of the rapid decrease being

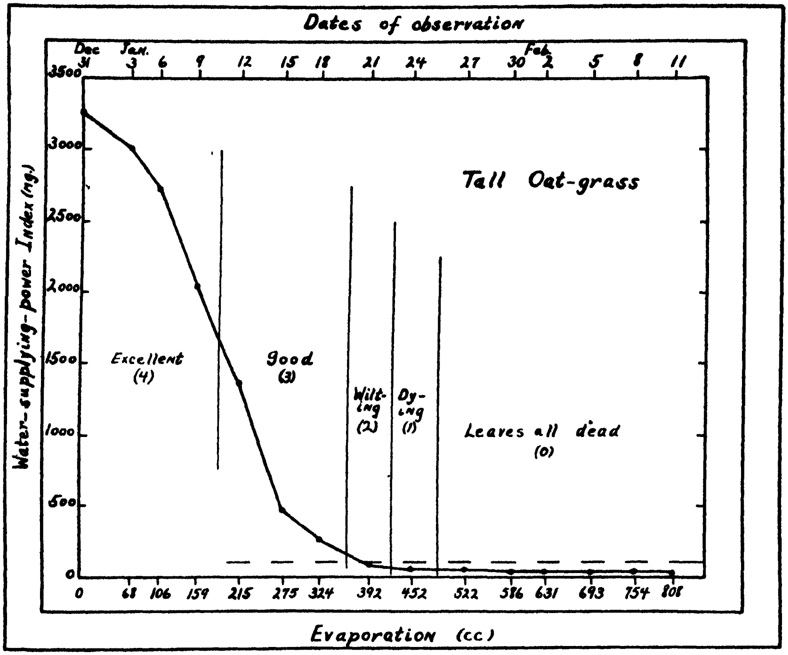

FIG. 2. Graph showing decrease in water-supplying power (ordinates) as in figure 1, but for soil with tall oat-grass. The area is divided into five parts representing five different conditions of health in the plants.

somewhat delayed in the case of the unplanted area but more prompt in the other cases, especially for the area planted with red fescue. For the ordinate range from about 3,000 to about 500 the graph approximates a straight line in each case; that is, the rate of decrease in the index value appears to have been approximately proportional to the accumulating total of evaporation from the blackened atmometer sphere. But since the evaporation total, measured at 3-day intervals, was almost proportional to the lapse of time, it is clear that the downward slope of the graph is also about proportional to the passage of time in the period of this most rapid decrease in the water-supplying power of the soil. With more frequent observations the question thus apparently raised (whether the graph slope in this region is to be considered as proportional to evaporation or to time) might have been answered, especially if the actual evaporation conditions had been less nearly the same for the three rooms. On *a priori* grounds it seems certain that the rate of evaporation, and of transpiration, rather than time itself should be regarded as the determining factor here. The slopes of these rectilinear parts of the four graphs are similar; the values of y/x being 12.3, 14.9, 15.0 and 13.3, respectively. The significance of the differences among these values, if they are significant, cannot be judged from the data at hand.

The direction of each graph changes beyond the lower end of the straight-line portion just considered and more or less gradually approaches the horizontal. This means that the rate of decrease in the water-supplying power of the soil, in relation to evaporation from the atmometer (and to time), becomes slower as the soil dries out, which would of course be expected; the water-supplying power cannot decrease very much after low values have already been reached. The slope of the graphs is very low after the supplying-power value of 100 has been passed.

The water-supplying power of the planted soil areas generally decreased somewhat more rapidly than did that for the area without plants. This was probably due to the fact that the plants with their leaves in the air and their roots ramifying and advancing through the soil mass, increased the effect produced by the evaporation conditions in removing water from the soil and in the consequent lowering of the water-supplying power.

The idea already advanced by LIVINGSTON and KOKETSU (**17**), by LIVINGSTON and OHGA (**19**), by WILSON (**32**), and by WELTON (**31**), that the water-supplying-power value of about 100 (for the 6-cm. depth) may be regarded as critical for many plants and for ordinary evaporation condi-

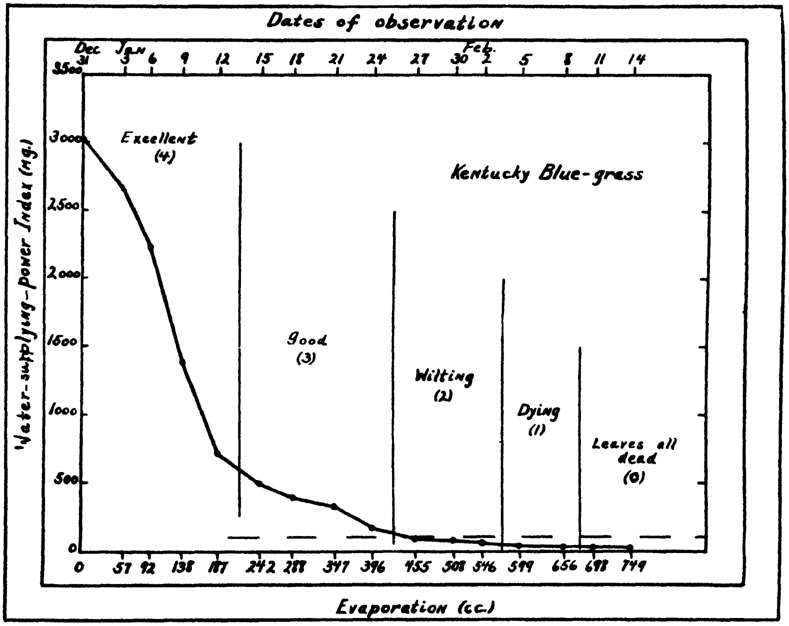

FIG. 3. Like figure 2, but for soil with Kentucky blue-grass.

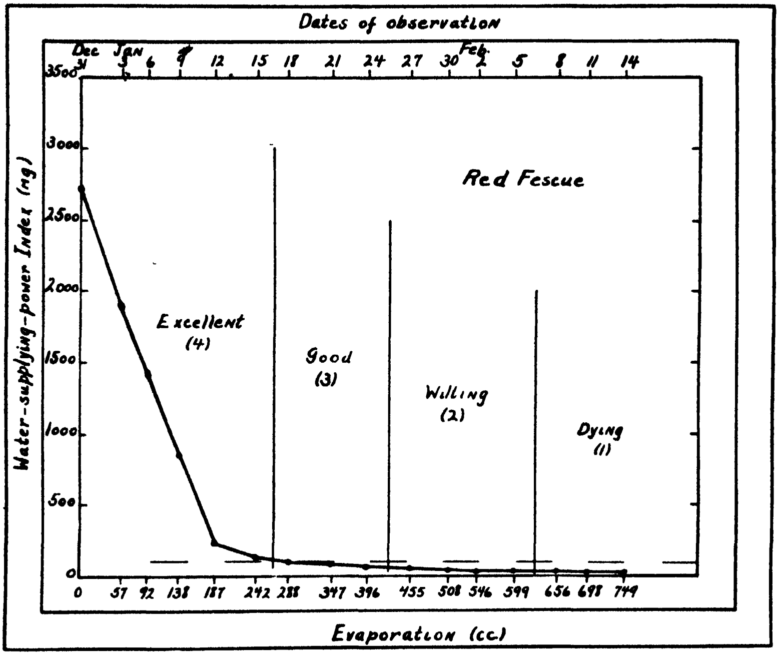

FIG. 4. Like figures 2 and 3, but for soil with red fescue. Note that all leaves were not yet dead at end of experiment.

tions, receives support from an inspection of figures 2 and 3 and table II. Several interesting points in this connection are brought out by an examination of the table.

There is only one case (that of red fescue) in which the index value for the water-supplying power of the soil was observed to be as low as 100 when the very beginning of growth retardation was first noted. In three cases (those of meadow fescue, Vermont bent grass and Washington bent grass) this critical value was first recorded six days after growth began to be retarded. Nine of the plants (tall oat-grass, orchard grass, brome grass, timothy, Bermuda grass, Chewing's fescue, redtop, Canada blue grass and sheep's fescue) showed the beginning of growth retardation nine days before the critical valúe was recorded. For four plants (Rhode Island bent grass, white clover, alsike clover and Kentucky blue grass) this period was twelve days. The period for the two remaining forms (velvet bent grass and perennial rye grass) was 15 days. Therefore, all but one (red fescue) of these nineteen plants began to show some slight effect of drouth much before the soil-moisture index had decreased to the value 100, but injury was not serious.

If we examine the table with reference to the soil-index values coincident with the beginning of definite injury (that is, with reference to the first boldface value given for each plant), we note that these values are below or about 100 for all forms. In the case of velvet bent grass serious injury was first observed with an index value of 106 and this is consequently not a significant exception to the general rule. In the case of meadow fescue, nothing worse than growth retardation was recorded for index values of 93 and 75, and definite injury first appeared only when the index value had decreased to 60. For sheep's fescue and red fescue the beginning of serious injury was recorded for index values of 48 and 52 respectively, while the beginning of growth retardation was shown for values of 475 and 99 respectively. All these fescues are well-known to be drouth-resistant. Their more wire-like leaves do not respond to drouth as promptly as do the ribbon-like leaves of most grasses.

The index value corresponding to pronounced injury (those marked P in the table) are all much less than 100, and the complete disappearance of bright green color (vegetative death) was first observed for values (marked D in the table) ranging from 52 to 35. The corresponding values for sheep's fescue and red fescue would have been somewhat below 27 and 29 respectively, but the experimental period was not prolonged sufficiently to bring about complete vegetative death in these two forms.

It therefore appears that a water-supplying power of 100 may be regarded as approximately and generally critical for these cultures. Had water been added so as to maintain the index value above 100 all of the plants would probably have continued to grow without very serious drouth injury. Nevertheless, it is clear that, while the value 100 appears to be a safe limit, below which the water-supplying power should not fall if vegetative health is to be maintained (for these plants and under these conditions of evaporation), yet the several plant forms differed markedly in their ability to withstand drouth.

A study of the graphs gives a clearer picture of the differences just considered than can easily be secured from the table. The vertical lines separating each graph (excepting that of the control area) into regions to show the five different growth conditions are each placed about the middle of the space representing an observation period, it being certain that the change in the plants from one condition to the next occurred between two consecutive observations. More frequent observations would, of course, have furnished data for the more accurate placing of these lines, but the error thus introduced can never be large. Supposing that each plant graph fairly represents the progress of its plants from excellent health to vegetative death, we may read from the graph the approximate water-supplying power of the soil for each of the points where the graph crosses the vertical lines. These supplying-power values may be considered as approximately marking the transition from excellent to good growth, from good growth to wilting, from wilting to dying and from dying to complete vegetative death. At the same time we may note, in addition, the total amount of evaporation from the black atmometer that corresponds to the position of each vertical line, these totals being read along the base of the graph. Each represents the summation or integration of all the evaporation influences, from the time of the last watering to the time of the occurrence of the particular plant change in question. Table III presents the results of such a study of all the graphs, including the three here shown in figures 2 to 4.

Although the data of table III are to be considered as only approximately representative of the actual occurrences in these greenhouse cultures, yet they appear to show clearly that there were marked differences among the plant forms with respect to these critical values of the water supplying power of the soil at 6-cm. depth. With reference to any one of these criteria some forms appear to have been more drouth resistant than others and the order of drouth resistance is not the same for the several criteria. Velvet bent grass appears to have shown the first effect of drouth with an index of water-supplying power as high as 2421, but this value is probably erratic; the first criterion is not easy to employ. Allowing for such instances, however, the possibility seems to be indicated that a plant shown to be *highly* drouth resistant with respect to the onset of vegetative death may be shown as of *low* drouth resistance with respect to the change from excellent to good growth or with reference to the onset of wilting. For example, sheep's fescue, which appears to be very drouth resistant on the basis of onset of vegetative death, is like Washington bent grass (920) with respect to the change from excellent growth to good growth, while the last-named form is not shown to be especially resistant by the criterion of vegetative death, 42.

It may well be that we have in such cases indication of a very important ecological and physiological principle; namely, that some plants may be highly sensitive to drouth conditions while they are still growing but exceptionally drouth resistant after growth has been stopped or after wilting has supervened through the influence of inadequate water relations. In comparing different plants with respect to their different degrees of drouth resistance, it is necessary to take into consideration the physiological criteria employed, as well as the kind of externally effective influences dealt with. Our sheep's fescue was apparently not very drouth resistant toward soil-moisture conditions while it was still growing, but it was exceptionally resistant after growth had ceased and many of its leaves had died.

Follet-Smith (5) in a summary of the water-supplying power of soils at the time of plant wilting, as ascertained by different investigators, found

TABLE III

APPROXIMATE CRITICAL VALUES OF THE WATER-SUPPLYING POWER OF THE SOIL AND OF TOTAL EVAPORATION FROM THE BLACK ATMOMETER (SINCE THE LAST WATERING), CORRESPONDING TO CHANGES IN THE CONDITION OF THE PLANTS DERIVED FROM GRAPHS FOR THE DATA GIVEN IN TABLES I AND II

NAME OF PLANT	FOR CHANGE FROM							
	EXCELLENT TO GOOD GROWTH		GOOD GROWTH TO WILTING		WILTING TO DYING		DYING TO VEGETATIVE DEATH	
	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION
	mg.	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*
Tall oat-grass	1700	186	178	358	75	422	55	487
Orchard grass	1730	186	142	358	70	422	50	487
Brome grass	1733	186	175	258	55	487	45	554
Rhode Island bent grass	1100	245	108	422	66	487	44	554
Velvet bent grass	2420	186	170	422	96	487	46	608
Meadow fescue	560	163	68	372	57	481	46	527
White clover	1470	176	130	346	53	471	43	585
Vermont bent grass	560	163	62	372	48	481	44	572
Timothy	1500	245	230	422	50	608	43	662
Washington bent grass	920	300	138	422	51	554	42	662
Bermuda grass	1090	245	92	487	53	610	41	662
Chewing's fescue	435	245	90	422	44	554	35	662
Alsike clover	1440	176	110	408	55	535	32	635
Redtop	1520	245	70	487	46	662	39	723
Kentucky blue grass	590	215	120	426	50	573	41	677
Perennial rye grass	1050	163	160	372	55	527	43	677
Canada blue grass	445	265	106	426	50	627	38	723
Sheep's fescue	920	245	54	487	38	508		
Red fescue	112	265	56	426	32	527		. .
Average	1121	216	119	405	55	527	43–	615+

this value to vary from 7.5 to 42, depending on the plants concerned and environmental conditions involved. The average value for this condition for the plant forms included in our table III was 55 and the value for a stage intermediate between wilting and vegetative death is 43. Our greenhouse values generally agree very well with those of Follet-Smith, although our averages are somewhat higher. Many of the individual values fall near the 42-mg. value.

The evaporation data given in table III indicate in a general way, as would be expected, that these evaporation totals increase as the indices of water-supplying power decrease. The averages shown at the bottom of the table are instructive in this connection. Of course, they represent all the plants taken together. The suggestion that evaporation totals accumulated since the last watering may be employed as rough indices of the water-supplying power of the soil, has been implied by many writers in ecology and it has been considered by Wilson (**32**) in some detail.

Another method for securing indices of drouth resistance as related to evaporation may consist in determining from the graph for each plant form the total amount of water lost from the blackened atmometer between the time when the water-supplying-power index first fell below 100 and the time when the plants passed into the condition of vegetative death. This amount of evaporation is represented by the horizontal distance measured on the 100-line, from the intersection of the graph with that line to the intersection of the same line with the vertical line that separates sections 1 and 0. These intercepts might be calculated directly from the numerical data but the graphical method is simpler and sufficiently precise. Their values for the 19 plants are listed below in the increasing order of their magnitudes. They are expressed as cubic centimeters of evaporation (corrected) from the Livingston standard blackened atmometer sphere.

Tall oat-grass	100	Kentucky blue-grass	237
Orchard grass	107	Meadow fescue	249
Rhode Island bent grass	124	Chewing's fescue	252
Velvet bent grass	138	Redtop	275
Brome grass	168	Perennial rye-grass	281
Washington bent grass	178	Canada blue-grass	290
White clover	208	Vermont bent grass	300
Bermuda grass	209	Sheep's fescue	458+
Alsike clover	210	Red fescue	465+
Timothy	212		

The values for sheep's fescue and red fescue must be greater than 458 and 465, respectively, which are the largest of our series.

Tall oat-grass is again shown as least drouth resistant and sheep's fescue and red fescue are again shown as most drouth resistant. It is interesting to note that very high drouth resistance was indicated for red fescue in the lawn studies reported by WILSON (**32**).

There seems to be no value of the water-supplying power that can correspond to "permanent wilting" for all these plant forms, although all had the same kind of soil and approximately the same evaporation and light conditions. This is, of course, just what would be expected from the physics of the soil-plant-air system and from the experimental studies on wilting points and soil moisture contents carried out by CALDWELL (**4**) and by SHIVE and LIVINGSTON (**26**). It becomes increasingly clear that the doctrine of unavailable soil moisture and wilting points, as set forth by BRIGGS and SHANTZ (**3**) and frequently accepted without question, is greatly in need of fundamental limitations based on still further critical study. VEIHMEYER and HENDRICKSON's recent studies (**29**) are important in this connection.

The apparently rather definite and significant relation between the decrease in the water-supplying-power of the soil and the corresponding increase in the total amount of water lost from the blackened atmometer sphere since the last watering, is consistent, in general, with a similar relation emphasized by WILSON from his studies on lawn plants in the open. He found that the index of the water-supplying power of his lawn soil (which has somewhat higher water-holding power than the one used in these greenhouse cultures) was about 2,000 shortly after a rain and decreased by about 4.6 for each atmometric increment of 1 cc., for the range of water-supplying power from about 2,000 to 100. It is interesting to secure corresponding ratio values for these greenhouse cultures, for comparison. The average period dealt with in WILSON's outdoor study was only 11 days in length and some rain occurred within the period in each case. In this greenhouse study, on the other hand, there was no irrigation or any addition of water to the soil during the period while the water-supplying power was decreasing from about 2,000 to about 100. Besides the unplanted area in the third greenhouse room, three planted areas, one from each greenhouse section, may be considered as representative; namely, those with alsike clover (room III), Kentucky blue grass (room II), and velvet bent grass (room I). The lengths of the periods in question for these four cases were 15, 21 and 15 days, respectively, for the planted areas, and 18 days for the unplanted area, the average length of period being 17 days. It will be noted that this period was considerably longer for the greenhouse cultures in winter than for the open lawn in summer, in spite of the fact that the period of the latter was not wholly without rain.

The supplying-power index of the alsike area was 1,947 on January 9 and it had fallen to 85 fifteen days later, on January 24—a decrease of 1,882. For this period the total loss from the blackened atmometer sphere in the same greenhouse room was 339 cc., and the index value consequently decreased at the average rate of 5.55 for each cubic centimeter of total evaporation. The area with Kentucky blue grass had a water-supplying power of 2,222 on January 6 and on January 27 this had fallen to 87, the total decrease being 2,135 in 21 days. This period showed an accumulated water loss from the blackened atmometer sphere of 398 cc. and the average decrease in the index value was 5.36 per cubic centimeter of total evaporation. The water-supplying power of the area planted to velvet bent grass was 2,011 on January 12 and on January 27 it was 86, the decrease being 1,925 in this case. The evaporation for the period of 15 days was 363 cc. and the average decrease in the supplying-power index was 5.30 per cubic centimeter of total evaporation. For these three planted areas which do not differ significantly in this respect, the average rate of decrease in the index value is 5.4, for the range from about 2,000 to about 100—more accurately to about 80.

The unplanted area had a water-supplying power of 2,123 on January 9, which decreased to 75 by January 27, thus showing a decrease of 2,048 in 18 days, and the corresponding total evaporation during this period was 407 cc. This gives a decrease in the soil-moisture index of 5.0 per cubic centimeter of total evaporation. Thus the mean rate of decrease in the water-supplying power for the range considered and for the 6-cm. depth, was somewhat less for the unplanted area than for any of these planted areas, as would be expected; since no water was lost from the soil of the former through plant absorption and transpiration. The difference between the average rate for the unplanted area and that for the three planted areas is only 4 in 54, or 7.4 per cent. This is a small difference but it may represent the influence of this kind of vegetation cover on the drying-out of the soil at the 6-cm. depth, under the climatic and soil condition of these greenhouse cultures. These mean decrements of water-supplying power for each cubic centimeter of corrected water loss from the blackened atmometer sphere may be summarized as follows: For the open lawn in summer WILSON (**32**), 4.6; for planted greenhouse flats in winter, 5.4; and for the unplanted greenhouse flat in winter, 5.0.

The rate of decrease in water-supplying power per cubic centimeter of evaporation from the standard blackened atmometer sphere was greater in all four cases in the greenhouse than in any case recorded by WILSON for the open lawn. This was to be expected, since no addition of water to the soil occurred in the greenhouse, while there were light rains during every period of drying-out in WILSON's study. Other differences may also be important, especially the difference between the two soils and between the components of the evaporation complex in the two studies, but both results are to be considered as only approximate.

From these and other similar considerations it seems possible that atmometer records might be employed in place of soil-point readings, to indicate the need of irrigation and the approach of injury from inadequate water supply during drouth periods, at least for such plants as are dealt with in this study. For example, for a soil similar to those here considered it might be presumed that about 300 or 350 cc. of water might be lost from a properly exposed standard blackened atmometer sphere between two consecutive applications of water, each application being sufficient to wet the soil to its maximum field capacity. Atmometer records would be kept by cumulative daily readings and a thorough watering would occur on the day following the one on which the total of all readings (since the last watering) amounted to 350 cc. Then the summing would be begun again, etc. Such a procedure would be comparatively simple and it should maintain the water-supplying power of the soil at a higher value for greater depths. At the same time there would be little chance of deficient soil aeration through too long continued high water contents of the soil. This suggestion has not yet been tested in practice, but it may have real practical value for those who desire to maintain healthy plant growth, as for a lawn throughout the growing season, with a minimum application of water and without any periods of considerable growth retardation or discoloration due to drouth. The summation value to be employed would doubtless vary with the nature of the soil used and it might be much greater for specially drouth-resistant grasses, such as red fescue and sheep's fescue, than for such forms as velvet bent grass or Washington bent grass. It might be safe to neglect rain unless it was quite certain that any period of precipitation had brought the soil-moisture index to 2,000 or above.

This new suggestion, that atmometers might be employed to furnish a possibly valuable guide to the moisture condition of the soil, without recourse to any soil measurements at all, is worthy of serious attention in tests at the hands of ecologists and agriculturists, agricultural climatologists and others interested in the ecological aspect of plant water relations. The suggestion appears to be specially promising for stations where little or no rain falls during the growing season and where water must be supplied to cultivated plants by means of irrigation, but the effect of precipitation, when it occurs, may be taken into account after some preliminary study. The critical value of total evaporation would, of course, vary with the kind of plants grown, with their stage or phase of development, with the water-holding power of the soil, with its depth and the depth to which the plant roots penetrate, etc. The principle involved may be stated as follows: For any given upland plant at any stage of its growth and for the particular soil in which it is rooted, the water-supplying power of the soil mass occupied by the root system should be adequate for healthy growth just after the soil has been thoroughly wetted by rain or irrigation and allowed to drain, and the water-supplying power should remain adequate for healthy growth until the continuously summed drying effect of the air and radiation conditions (as these operate to remove water from the soil by direct evaporation and by plant absorption and transpiration) has attained a certain critical value. It seems likely that the continuous summation of daily readings from a suitable form of atmometer suitably exposed may give a satisfactory measure of the summed drying influences and that such an atmometric summation may indicate approximately when the water-supplying power of the soil approaches a point where it would be inadequate for satisfactory growth of the plant.

Pot cultures

A few tests with potted broad-leaved plants were made in connection with the experiments already described. These will now be presented briefly. Ordinary 6-in. pots were used. The plant forms employed were buckwheat (*Fagopyrum esculentum* Gaertn.), kidney bean (*Phaseolus vulgaris* L.), *Coleus blumei* Benth., hollyhock (*Malva sylvestris* L.), and sensitive plant (*Mimosa pudica* L.). The cultures were started late in September, 1925, all with a mixture of sand, loam and humus like that used for the cultures in flats. Fifteen pots were prepared for each kind of plant, with five plants in each pot for buckwheat, four for bean, and one for each of the other forms. Some additional pots of buckwheat had fifteen plants each. The pots were watered at intervals until vigorous plants were secured. The last watering for buckwheat, bean and Coleus occurred on November 16, buckwheat and bean being then in bloom, and watering was discontinued April 9 for hollyhock and sensitive plant. The cultures were allowed to dry out gradually after watering had ceased, and soil-moisture determinations were made at the beginning of visible wilting and at two subsequent stages of drouth injury. The numerical results were recorded in terms of water content of the soil per unit of natural volume, as well as in terms of the water-supplying-power value determined by soil points for the 6-cm. depth and for the 1-hr. period. To determine the water content three cork-borer samples, 1 cm. in diameter and 7 cm. in depth, were taken for each test and these three were combined to form a composite sample. The resulting water-content value represents an average for the pot. Similarly, three soil points were used in each pot at each observation, the average reading being recorded. It should be noted that evaporation from the pot walls as well as from the soil surface accelerated the drying-out of the soil, and wilting occurred sooner than would have been the case had the containers been impervious to water.

The last two columns of table IV present the results of these tests when the plants were just beginning to wilt, when they were badly wilted and when the leaves had just become thoroughly dried. (What is here termed "bad wilting" may be considered as approximately the stage called the beginning of permanent wilting by BRIGGS and SHANTZ, though no recovery tests were carried out on these pot cultures excepting for some of the pots of bean.) At the bottom of the table are given corresponding data for Coleus and hollyhock when in good condition, shortly before these plants began to wilt. The number of plants in a pot and the number of pots tested in each case are also shown in the table.

TABLE IV

SOIL-MOISTURE DATA FOR POTTED PLANTS IN THE GREENHOUSE

CONDITION OF PLANTS	PLANT NAME	NO. OF PLANTS IN POT	NO. OF POTS TESTED	WATER-SUPPLYING POWER OF SOIL AT 6-CM. DEPTH	AV. WATER CONTENT IN 100 CC. OF SOIL
Plants just beginning to wilt	Buckwheat	15	3	50	5.46
	Buckwheat	5	3	48	6.08
	Bean	4	3	42	5.16
	Coleus	1	3	40	5.47
	Hollyhock	1	4	43	5.36
	Sensitive plant	1	8	44	6.48
Plants badly wilted	Buckwheat	5	6	36	4.60
	Bean	4	6	34	5.05
	Coleus	1	3	36	5.47
	Hollyhock	1	4	32	4.16
	Sensitive plant	1	3	23	3.88
Leaves dried up	Buckwheat	5	3	27	4.03
	Bean	4	3	26	4.25
	Coleus	1	3	29	4.20
Plants in good condition	Coleus	1	3	164	8.26
	Hollyhock	1	3	60	7.73

Since the observation of the second and third stages of drouth injury here dealt with was not so definite as that of the beginning of wilting, the most interesting values to study are those of the first section of table IV. In buckwheat, bean and Coleus wilting begins as a noticeable drooping of the leaf blades. In hollyhock the first sign of wilting is a flattening of the blade, previously somewhat concave upwards. The ultimate leaflets of the sensitive plant fold at the beginning of wilting as they do in assuming their night position or as a result of mechanical stimulation. The use of this definite response has been emphasized by KOKETSU (**10**) as a criterion for fixing upon an exceptionally precise stage of wilting in this plant.

For all these plants the beginning of wilting occurred with a water-supplying power between 50 and 40 and with a water content between 5.15 and 6.48 per cent. (on the basis of natural volume). From the data for water-supplying power it appears that the buckwheat plants required a significantly higher value (48 or 50) to prevent the onset of wilting than was required by the other forms and that the latter were so nearly alike with respect to the water-supplying power of the soil at the beginning of wilting that the differences indicated (between 40 and 44) may be considered as insignificant. The value for the sensitive plant (44) is probably somewhat more reliable than the values for bean, Coleus and hollyhock, because of the larger number of tests involved in the average for the sensitive plant as well as because the beginning of wilting was probably more precisely observed in that case than in the others. Whether fifteen or five plants of buckwheat were present in the 6-in. pot made no significant difference in the soil-point value corresponding to the beginning of wilting. It may be noted that the soil point readings at the time of bad wilting for the five species listed in the second section of the table all fall within the limits of 7.5 to 42 mg. mentioned by FOLLET-SMITH (5) in his summary of the wilting point values obtained by various workers. Nevertheless, the soil-point values given for successive stages of drouth injury for each of the other forms are remarkably consistent and indicate such differences as might be expected. The water-content data are not so consistent, however.

It appears in general that both water-supplying power and water content are to be regarded as useful for studying changes in soil-moisture conditions between two successive stages of drouth injury, but that the soil-point method is apt to be somewhat more sensitive and probably more precise. The soil-point method gives promise of great advantages when the soils of a series are not nearly alike, but those advantages are not shown in this series because the same kind of soil was used for all cultures.

From the data of table IV, buckwheat appears to have shown about the same degree of drouth resistance as was shown by bean and Coleus if we use the second or third stage of injury, while it was less resistant than the other two forms if we use the first stage of injury. Bean, Coleus, hollyhock and sensitive plant appear to have shown about the same degree of drouth resistance on the basis of the beginning of wilting but the sensitive plant showed a much higher degree of drouth resistance than any of the others on the basis of the onset of bad wilting. Such differences between the indications secured for different degrees or stages of water deficit may be partly due to uncertainty in observing the exact stage desired in any instance, but it seems reasonable to suppose that different stages of wilting may not agree in their indications as to drouth resistance, as has been said in the earlier part of this paper.

The results secured from these pot tests cannot be compared in detail with those from the cultures in flats, described in the first part of this paper, notably because the period of drying-out of the soil mass was much shorter in the case of the pot tests. Evaporation conditions for the two series were somewhat different also, and these should influence the results. In a general way, the two series agree in showing the remarkable sensitiveness of the soil points when compared with the plants and in furnishing evidence of the promise and practicability of the soil-point method for studying soil-moisture conditions.

Conclusion and summary

The water-supplying power of the soil adjacent to plant roots is the single dynamic soil feature that directly affects plant water relations. All other soil features or characteristics that appear to influence plant activity through water relations are indirect in their operation, for they either affect the water-supplying power of the soil (directly or indirectly) or else they affect the aerial environmental complex or the capacity of the plant itself to absorb or conduct or retain water. The water-supplying power of a soil may be directly measured by the soil-point method, without reference to the kind of soil used, its degree of packing or its water content. The numerical values or indices secured represent the ability of the soil to deliver water to an absorbing surface. The standard instrument has an absorbing area of 12 sq. cm. and the standard period of its exposure in the soil is 1 hour. If any index value is divided by 12 the resulting quotient is the hourly rate of water delivery by soil to soil point across an area of 1 sq. cm., for the hour of the test.

This study of greenhouse cultures in winter (carried out at the Johns Hopkins Laboratory of Plant Physiology, in Baltimore) deals with some of the more easily detected relations that exist between (a) soil-moisture conditions as represented by the water-supplying power of the soil at a depth of 6 cm. and (b) plant health and vigor as these alter with the drying-out of the soil mass after the cessation of watering. As this drying-out progresses the plants first show growth retardation, followed by complete cessation of growth. They then wilt and finally die by degrees, the leaves being, of course, the first parts to be visibly affected.

For any particular soil mass the water-supplying power at any particular place in the mass is specific for any given instant. It is greater the greater is the water content and it is different for different degrees of packing. An upland soil with water content equal to its maximum field capacity (as when downward flow of water has just ceased after a heavy rain or after the artificial application of water) can supply water to an absorbing surface, such as that of root, root-hair or soil point, at a rapid rate; but the supplying power steadily decreases as the soil dries out through absorption of water by plant roots and through evaporation into the air above. The water films become thinner, the resistance to capillary movement increases and the capacity of the soil to supply water to a root system eventually becomes insufficient to maintain health in a plant with stationary root surfaces. However, plants generally extend their root systems continually into new soil regions, where the water content and water-supplying power have been less depleted. When the combined effects of root advance and capillary movement of water fail to supply water at a rate sufficient to make up for the loss and use of water by the plant (transpiration, tissue enlargement, etc.), then the water content of the plant begins to decrease and wilting soon begins. If no water is added to the soil adjacent to the root system, wilting becomes more pronounced and extensive and withering and death eventually supervene. In the meantime, the water-supplying power of the soil region occupied by the root system continues to decrease. Although it may be true that the production of root branches and root-hairs is generally, or, in some cases, most vigorous in soils with supplying powers somewhat below the optimum for most active top growth, nevertheless pronounced wilting and withering of the leaves is probably accompanied by great retardation or cessation of root growth. For any short interval in the drying-out period there appears to be a fairly definite relation between the health of the plant and the current magnitude of the water-supplying power of the soil about its roots, if we consider other influential conditions as remaining practically unchanged.

This paper deals mainly with (a) the progressive decrease in the initial water-supplying power of the soil (measured by means of soil points applied for one hour at the 6-cm. depth) as the soil dries out after watering has been discontinued, and with (b) the accompanying gradual change in the condition of the plants as they pass from excellent to good health, from good health to the beginning of wilting, from the onset of wilting to the occurrence of bad wilting, and from the onset of bad wilting to the onset of vegetative death, (when all leaves are withered). The occurrence of ''bad wilting'' in our cultures corresponds closely to the onset of ''permanent wilting'' as roughly defined by BRIGGS and SHANTZ.

In the main series of experiments, greenhouse cultures in wooden boxes or flats were employed. There were seventeen different grasses and two clovers, also three control soil areas without plants. The same kind of soil was used for all flats, a light loam. When a well-established turf had been formed in all cultures the soil was thoroughly wetted for the last time and watering was discontinued. Throughout the ensuing period of the drying-out of the cultures records of plant condition and of the water-supplying power of the soil were made at frequent intervals and corresponding records were kept of air temperature and of evaporation and water loss from standardized LIVINGSTON spherical atmometers, white and blackened with lampblack. The indices of water-supplying power are expressed in terms of milligrams of water absorbed by a single standard soil point (about 12 sq. cm. of absorbing surface) in one hour, at a depth of 6 cm. beneath the top of the soil mass.

The numerical data are given in tables, and sample graphs are presented for the control soil areas without plants and for the cultures of tall oat-grass, Kentucky blue-grass and red fescue. The numerical value for the water-supplying power of the soil at the 6-cm. depth decreased from about 3,000 to about 500 in nearly the same manner in all the flats. After the first few days the rate of decrease was nearly uniform for a long period and about proportional to the lapse of time. When the index value had reached about 500 the rate of decrease became slower, being very slow for values below 100.

In twelve out of the nineteen plant forms studied actual drouth injury became apparent about the time that the decreasing water-supplying power of the soil attained the critical value of 100. For the remaining seven forms injury did not appear until still lower values had been reached.

Of these nineteen species, tall oat-grass showed the lowest degree of drouth resistance; its leaves were all dead by the time the index of the water-supplying power of the soil had decreased to 52. Red fescue and sheep's fescue showed the highest resistance; they still had many living leaves when the index value had decreased to below 30. At the end of the experiment period of about six weeks, with no water added to the soil and with a total evaporation of about 800 cc. for the period (from the blackened atmometer sphere), all of the leaves were dead for all forms except sheep's fescue and red fescue.

Clear relations were shown between the rate of decrease in the water-supplying power of the soil and the corresponding rate of water loss from the blackened atmometer. For the period during which the value of the water-supplying power was decreasing from about 2,000 to about 100 this relation was about the same for all soil areas; the index value was diminished, on the average, by 5.4 (in the case of the planted flats) or by 5.0 (in the case of the control area) for each cubic centimeter of total evaporation. These ratio values are somewhat greater than a corresponding value (4.6) found by WILSON in his studies on the soil of an open lawn in summer at Baltimore.

It is suggested that the total evaporation that has occurred from a suitably exposed standard atmometer since the last preceding considerable application of water to the soil may offer a means of approximating the water-supplying power of a field soil at any time within a rainless or nearly rainless period. If the losses from the atmometer are summed from day to day, as such a drouth period advances, it appears from our studies and from that of WILSON that the summation amounts to about 350 cc. or 400 cc. at the time such plants as lawn grasses begin to show drouth effects. The critical evaporation total should depend on the kind of soil, kinds of plants, etc., and upon the daily rate of evaporation. It should be different for sufficiently different climatic complexes.

The several cultures did not indicate a uniform relation between amounts of total evaporation (since the last watering) and the onset of vegetative death in the plants. Beginning when the soil-moisture index was about 100, vegetative death occurred for tall oat-grass when only 100 cc. of water had been lost from the blackened atmometer sphere. For Canada blue-grass this evaporation total was 290, while for sheep's fescue and red fescue vegetative death had not occurred with evaporation totals of 458 and 465, respectively.

A few experiments were performed with pot cultures (in ordinary 6-in. pots in the greenhouse, in winter and spring) of buckwheat, Coleus, sensitive plant, bean and hollyhock. These were treated about like the cultures in flats, having the same kind of soil as those, but records were made of volumetric soil-moisture content as well as the soil-point readings, at intervals during the drying-out of the soil following the last watering. These two methods for securing numerical indices of soil-moisture conditions generally agreed very closely. Since the soils were alike for all cultures the two methods should be about equally useful in such a series, but the dynamic method appears to be somewhat more precise.

The beginning of wilting occurred, for all five of the potted forms, with supplying-power values between 50 and 40, and with volumetric residual water contents of the soil between 6.48 and 5.15 per cent. Buckwheat appeared to be least drouth-resistant by these criteria and the other plants were apparently somewhat more resistant and about alike. Hollyhock and sensitive plant were found to be specially suitable for studies of this type, because of their delicate and easily observed leaf responses at the beginning of wilting.

OHIO AGRICULTURAL EXPERIMENT STATION,
THE JOHNS HOPKINS UNIVERSITY.

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AVAILABLE WATER AND THE WILTING OF PLANTS

ARTHUR D. MOINAT

Soil moisture in relation to plant growth is expressed upon the basis of its availability to the plant. Botanical or agricultural papers in which comparisons are made of the effects of different soils and soil moistures upon the growth of plants must necessarily consider the ability of the plant to obtain the moisture present in the particular soil. It is apparent however to those who have given such problems some study that our methods of determining the availability of water in a given soil are not very accurate nor very easily applied. It was with the view in mind of studying the plant in wilting and to improve, if possible, some of the methods used in investigating the water relations at wilting that this work was undertaken.

SACHS (**9**) as early as 1859 demonstrated the importance of the textural properties of the soil in determining the percentage of soil moisture which is available to plants. BRIGGS and SHANTZ (**1, 2, 3**) designated that percentage of moisture which remains in the soil at permanent wilting as the wilting coefficient. Permanent wilting was defined as the stage of wilting of a plant from which it fails to revive within 24 hours if placed in a saturated atmosphere. From their results they concluded that the wilting coefficient varies with the character of the soil but is very nearly the same for all types of plants. The differences found when different species were used were interpreted as being due to variations in root distribution. They were led to conclude further that the soil moistures at which plants are grown have no effect upon the value of the wilting coefficient.

VEIHMEYER and HENDRICKSON (**14**) have found evidence that plants are able to reduce the moisture content of different soils to different degrees of "dryness" before permanent wilting sets in. Therefore they believe that the wilting coefficient cannot be calculated from the moisture equivalent as is commonly done.

The soil has a water-retaining power at the wilting coefficient of 3 to 4 atm. according to SHULL'S (**10**) calculations on the basis of water intake of cocklebur seeds. The average osmotic pressure of the plant root is equivalent to 7 or 8 atm. as determined by HANNIG (**5**). However according to URSPRUNG (**12**) the average effective osmotic pressure is more likely to fall to 3 or 4 atm. Even with unimpaired ability of the root to absorb moisture the practical cessation of movement of water in the soil at low moisture ranges would cut down water intake to an entirely inadequate rate.

URSPRUNG and BLUM (**13**) have shown that the effective osmotic pressure of roots may vary by several atmospheres in a relatively short time. For example when *Vicia faba* was transferred from tap water to a 0.2N solution of sucrose and with the removal of the greater part of the root system, the remaining roots increased in effective osmotic pressure from 5.3 to 6.3 atm. RENNER (**7**) found that *Phaseolus* seedlings previously adapted to weak KNO_3 solution were able to absorb water from 3–5 per cent. KNO_3 solutions which represent 12–20 atm. of pressure respectively. RICOME (**8**) was able to cause the rapid wilting of *Vicia faba* and *Ricinus* plants when transferred from Knop's solution to Knop's solution with 1 per cent. NaCl.

Comparatively few investigators have studied the *plant* at permanent wilting. CALDWELL (**4**) found a decrease of 30 per cent. in the water content of the leaves of plants at permanent wilting. The percentage of water in leaves at permanent wilting was approximately constant for all individuals of the same species at the same stage and degree of development. This constant was unaffected by external conditions under which the wilting occurred. The water of the stem tissue was not considered, since according to this author, the water of the leaves of six weeks' old *Phaseolus* plants is much greater proportionally than that found in the stems. KOKETSU (**6**) found this constant (*i.e.*, the water present in the leaves at permanent wilting) to be affected by the character of the soil. With *Glycine* it was found that the higher the water-holding capacity of the soil the larger the critical water content of the leaves. Opposite results were obtained with *Coleus*. The ratio of the critical water content at permanent wilting to the water content at full turgidity was found to be higher for *Coleus* than for *Glycine* or *Mimosa*. According to KOKETSU, the lower one finds this ratio to be, the more xeric the plant.

Materials and methods

Seedlings of *Phaseolus vulgaris* (Burpee's Stringless Greenpod) as grown under greenhouse conditions were used in all the experiments. In most cases the plants were grown in brown silt loam soil. In a few instances sand cultures (silica sand) were used. The calculated wilting coefficient for the sand was 0.213 per cent. and that for the soil was 17.27 per cent.

In preliminary experiments direct determinations of the wilting coefficient of the soil were made according to the method of BRIGGS and SHANTZ (**1**). Plants were grown in soil about 1 inch deep, both with and without a wax seal. Each culture was brought up to its original weight and moisture content daily or every second day until the plants had reached the stage at which water was no longer applied. Soil samples were taken at permanent wilting from the deeper soil in which actively absorbing roots were located. All soil samples were dried to a constant weight at 100° C. and the per cent. of moisture calculated on the basis of the dry weight.

Other experiments were set up with the cultures growing in auto-irrigators in order to maintain a more nearly constant soil moisture. A description of this irrigator is to be published as a separate paper. There was space for nine soil containers in an irrigator; three of the containers were maintained at a soil moisture of approximately 15 per cent., three others were at approximately 26 per cent. and the remaining containers were held at a soil moisture of about 55 per cent. The soil moisture in a given container varied somewhat with different irrigators but was approximately constant throughout a single experiment. Six plants were grown in the soil of each container.

In starting a group of plants in an irrigator the seed was allowed to begin its germination in wet sand. It was then transferred to the soil, discarding at the same time all unsatisfactory seeds. The soil in all the containers of an irrigator was held at approximately 27 to 30 per cent. of moisture for three or four days or until the seedlings began to push through the soil. Soon thereafter the soil moistures changed to the percentages shown in the tables.

It will be noticed in several of the tables to follow that in certain cases the soil moistures at which the plants were grown were below the wilting coefficient. Due to the construction of the irrigator it was impossible to get a sample of soil which was representative of the moisture supply upon which the plants were depending except in those cases where the soil moisture was somewhat above the wilting coefficient.

In certain experiments the plants thus grown were subjected to permanent wilting and the per cent. of water present in the wilted plant determined. Permanent wilting was considered to be that state of wilting from which a plant will not regain the turgidity of its leaves if placed in the dark in a saturated atmosphere for a period of 24 hours. To determine the per cent. of water present in plant tissue, the material was collected in the fresh state in small closed containers, weighed, dried for 3 days at 100° C. and reweighed.

Plants grown at the different soil moistures provided by the system of automatic moisture control were used in tests in which the dry weight per unit of leaf area was measured. The leaf areas were determined with the aid of planimeter tracings of blue-printed or pencil-traced leaf margins.

In order to make the various tests more uniform the plants were not used until they had reached a definite stage of development. The stage most used was that in which the seedling had developed 2 well expanded, simple leaves (its first true leaves) and the internode above them was not over 5 mm. in length. The cotyledons at this stage were badly shrivelled but had not fallen off. In other plants the per cent. of water was determined when every leaflet of the first compound leaf was over 2.5 cm. in length.

To compare the wilting of plants grown in soil with that of plants grown in water cultures, a number of bean seedlings were grown in tap water in gallon earthenware jars. The water was changed daily in order to give more favorable conditions of growth. When the young plants had attained growth stages similar to those used in the tests just described, they were transferred to tumblers of tap water plus sodium chloride in various concentrations some of which were sufficient to produce wilting. The rate of wilting was increased in certain cases by means of air currents from an electric fan.

Results and conclusions

By reference to table I it is seen that direct determinations of the wilting coefficient gave values slightly lower than the values calculated from the moisture equivalent. This may have been due to certain properties of the soil used or to variations in the methods from those of Briggs and Shantz. A serious difficulty which is always apparent in the direct determination is the wide difference in the rate of wilting of individual plants growing in the same container.

TABLE I

Direct determinations of the wilting coefficient of silica sand and brown silt loam soil
Plants grown at the soil moistures indicated

Number of determinations	Original moisture content of soil	Moisture in soil at permanent wilting
	a. Results with silica sand	
		per cent.
3*	8% of the dry weight	0.047 ± 0.005
5*	11% " " " "	0.048 ± 0.004
5*	13% " " " "	0.115 ± 0.021
	b. Brown silt loam soil	
5†	32% of the dry weight	15.98 ± 0.11
7†	40% " " " "	17.20 ± 0.18

Wilting coefficient of sand calculated on the moisture equivalent – 0.213 per cent.
Wilting coefficient of soil calculated on the moisture equivalent – 17.27 per cent.
* Duplicated samples.
† Triplicated samples.

Plants which were growing in sand could be wilted very rapidly by withholding water. In soil cultures the wilting was less rapid, being an especially slow and gradual process when the soil surface was sealed over with wax. Seedlings growing in a soil culture which dries out very gradually develop a smaller leaf surface, a dark green color and gradually acquire more and more of the characteristics of xeric plants. It is highly probable that in this process the per cent. of bound water increases and perhaps there are cellular changes which are as yet little understood but which may be important from the standpoint of the wilting of the plant.

In both the sand and the soil cultures (table I), which were set up in shallow containers and watered at the surface from day to day to bring the culture up to the required moisture percentage, the containers in which a higher soil moisture was maintained previous to wilting, gave a higher wilting coefficient. The determinations were rather few but the tendency is in the direction which one would expect if a somewhat more xeric type of plant develops in a substrate maintained at a lower moisture content.

In order to study the variation in water present in a plant at permanent wilting a number of seedlings were grown under constant automatic water supply. In bringing the plants to permanent wilting they were treated in three different ways: (1) plants were wilted in the soil in which they grew by removing the container from the irrigator; (2) plants were removed from the soil without mutilation of the roots and allowed to dry on a wooden drying rack; (3) seedlings were cut at the surface of the soil and wilted upon the drying rack. The method by which the plants were brought to the state of permanent wilting had but little effect upon the water content of the plant when it reached that stage. However the rate of wilting was changed considerably according to the method used. The seedlings wilted very rapidly when they were removed from the soil with the root system left attached; they wilted less rapidly when cut at the soil, the cut surface having been waxed over, and finally the greatest time was required to wilt those plants which were left in the soil.

The data in table II, trial numbers 1–6 inclusive, indicate that, generally, the water held in the plant when it reaches the state of permanent wilting is greater if the plant has been growing at the higher percentages of soil moisture. This seems to occur in spite of the fact that the more xeric type of plant would tend to retain more bound water.

Wide variations in the rate of wilting of individual plants occurred throughout these tests. The differences were least evident in plants removed from the soil and wilted without removal of the root system. Where the seedlings were cut at the soil surface or in trials in which the plants were wilted without removal from the soil, different plants showed considerable variation in the rate at which they reached the state of permanent wilting. In certain cut plants the time required for wilting was 3 or 4 times the average. Uneven distribution of soil moisture might account for

TABLE II

MOISTURE CONTENT OF PLANTS AT PERMANENT WILTING

PLANTS WITH SIMPLE LEAVES			PLANTS WITH FIRST COMPOUND LEAF		
TRIAL NO.	SOIL MOISTURE	AVERAGE MOISTURE CONTENT*	TRIAL NO.	SOIL MOISTURE	AVERAGE MOISTURE CONTENT*
	per cent.	*per cent.*		*per cent.*	*per cent.*
1	56	735	7	56	759
	40	743		50	585
	20	704		49	688
				13	740
2	42	997	8	53	724
	19	865		46	628
	12	628		11	605
3	52	1070	9	46	628
	42	1026		43	683
	13	728		41	612
				17	620
4	53	926			
	28	829	10	54	655
	15	742		12	575
5	49	943			
	24	771	11	51	680
	13	731		44	581
6	15	692			
	15	696			
	10	639			

* Each percentage represents the average of from 6–9 determinations.

variations in the wilting rate of plants remaining in the soil but could only indirectly affect the rate in cut plants.

The data of table II illustrate the wide variation in the amount of water present in different sets of plants when brought to the state of permanent wilting. For example, the figures in trial 1 may be compared with those of trial 3, the two groups of plants having been grown under similar soil moisture conditions. The two upper soil moistures were sufficiently high to give an abundant supply of water to the plants and no great difference was apparent in leaf characteristics. Plants grown at the lowest soil moisture, that is, 10 to 20 per cent., were quite different from the others. One of the most important causes of the lack of consistency in the data lies in the difficulty of judging the wilting, especially if the plants were brought to permanent wilting on different days.

The per cent. of water present in older plants at the stage of permanent wilting is shown in table II, trials 7–11. These plants had developed the first compound leaf before they were subjected to the conditions of wilting. The data given do not show a consistently lower water content at permanent wilting in the case of the plants grown at a lower soil moisture. The youngest compound leaf is always the last to wilt; it is also the most difficult to detect wilting in due to its size and the presence of a large number of epidermal hairs. Since in the seedling stage a new leaf is continually developing while the last one is enlarging, the determination of permanent wilting is interfered with.

The determination of the "end point" of permanent wilting is made more complex by the fact that single plants which had apparently reached permanent wilting would often revive as many as three or four times after short exposures to the saturated atmosphere of a moist chamber. In other words, in a dry atmosphere the losses of water from the leaves were too rapid to be covered by the movement of water from the regions of storage in the hypocotyl. Seedlings which were wilted without the removal of the roots from the soil and especially those which were cut at the soil surface were quite likely to regain the turgor of their leaves if placed in a moist chamber. The groups of plants which were removed from the soil without mutilation of the root system wilted more rapidly than did the plants given the other treatments; they were also less likely to revive in the moist chamber. The seedlings grown in a dry soil, in many cases failed to revive because of a low per cent. of stored water.

The degree of drooping which a leaf displays, such as is characteristic of permanent wilting, may be indicative of a fairly narrow range of water content in the tissues of the leaf blade but it does not tell much with regard to the per cent. of water present in other parts of the plant. The more nearly permanent the wilting becomes the closer is the approach to a constant and similar water content in all the organs of the plant. It is to be expected, therefore, that determinations of leaf moistures at the critical point of wilting would be more consistent than would determinations for the entire plant.

Analyses of different regions of the plant gave proof that the hypocotyl is an important water storing organ of the bean seedling. Uprooted plants were exposed to dry air for 0, 0.5, 1, 2, 3 and 4 hours, then cut into 5-cm. sections for the determination of the percentage of water present. Table III gives the water content of the root and of the proximal (number 1) and successive 5-cm. sections of the shoot. Corresponding sections from two or three plants were weighed together in the same weighing bottle. All of

TABLE III

Moisture determinations on 5-cm. sections of seedlings
Uprooted plants exposed to air for periods indicated previous to cutting into sections

Period of exposure	Section of plant	Average moisture content*	Period of exposure	Section of plant	Average moisture content*
		per cent.			*per cent.*
None	Roots	953	2 hours	Roots	167
	1	1168		1	961
	2	1018		2	1090
	3	963		3	836
	4	899		4	936
	5	963		5	681
	6	957			
30 min.	Roots	577	3 hours	Roots	140
	1	1205		1	999
	2	1085		2	1006
	3	1133		3	931
	4	703		4	1018
	5	798		5	802
1 hour	Roots	154	4 hours	Roots	122
	1	1184		1	1074
	2	948		2	913
	3	873		3	814
	4	795		4	
	5	638		5	326

* Each percentage represents the average of 3 determinations.

the plants used had begun to develop the first compound leaf. The effect of different periods of wilting upon uprooted plants is shown in the table. The check plants were weighed immediately upon removal from the sand culture; other plants were wilted for the periods shown.

Beginning at the lower end of the stem a decreasing gradient of the water present is apparent in successive regions up the stem. The plants which were wilted for two or for three hours give some evidence at least that in wilting the root draws upon water present in the hypocotyl. The very uppermost leaves are the last to wilt in spite of the fact that the upper part of the stem is constantly losing water very rapidly and usually shows a lower water content.

That the degree of wilting may not necessarily be a criterion of the moisture content of the plant is indicated as possible by the data in table IV. It is shown that the dry weight per unit of leaf area is distinctly greater for plants grown in the drier soil. The difference is more significant in the case of the younger plants with only simple leaves. The greater dry weight per unit of area occurs with the development of numerous veins, numerous small cells with smaller vacuoles and less water. The increased amount of mechanical tissue supporting the leaf blade of the dry soil plant made necessary a greater loss of water to produce an equal drooping of the leaves. TUMANOV (**11**) found that leaves acquired a xeromorphic structure with more mechanical tissue present if the plants were subjected to alternate periods of optimal and sub-optimal soil moisture. The data in table IV explain in part the correlation between soil moisture and the quantity of water present at permanent wilting as given in table II. Thus the dry soil plants being slow to show signs of wilting have time to lose considerable stored water before they would be judged as permanently wilted.

TABLE IV

DRY WEIGHT OF LEAF TISSUE PER SQUARE CENTIMETER OF LEAF AREA

SOIL MOISTURE	NUMBER OF LEAVES	AVERAGE WEIGHT PER SQ. CM. LEAF AREA
per cent.	Simple leaves	*gm.* × 10^{-6}
50	16	1628 ± 21.6
25	16	1685 ± 24.8
19	8	2128 ± 61.7
16	16	2196 ± 27.5
12	8	4333 ± 45.4
	Compound leaves	
42	7	1745 ± 35.9
22	4	1442 ± 44.2
11	2	2098 ± 28.8

The fact that the leafy top of the seedling depends upon stored water in the hypocotyl when the moisture supply from the soil is cut off would lead to the conclusion that the critical point is reached in the soil (*i.e.*, absorption by the root falls to a very low value) some time before wilting is evident. Absorption at a low rate is continuous whether the plant is depending upon stored water to maintain leaf turgidity or has become permanently wilted. Without a wax seal, evaporation may cause more depletion of the soil moisture and the final result may be that the wilting coefficient determined upon the basis of the permanent wilting of the leaves may give a value somewhat lower than the true value for the critical state in the soil. Furthermore the plant with a xeromorphic leaf structure could use the soil moisture for a longer time before permanent wilting sets in than could a plant grown under more nearly optimal conditions of soil moisture. The results of the direct determinations of the wilting coefficient (table I) support this view.

For comparison with the soil cultures, plants were wilted in water cultures by transferring plants which had been growing in tap water to solutions of tap water plus various quantities of sodium chloride. Table V shows the results with bean seedlings transferred at the stage when two simple leaves were expanded. It is evident from the data that a concentration of sodium chloride between 0.07 M. and 0.08 M. representing 2.94–3.35 atm. pressure is barely strong enough to reduce the absorption sufficiently to cause wilting. A solution of 3.76–4.15 atm. (0.09 M.–0.10 M.) seems to just balance the effective osmotic pressure developed in the roots of the plant. The time required to produce wilting varied with the concentration of the salt solution and the evaporating power of the air. An hour's ex-

TABLE V

DEGREE OF WILTING OF SEEDLINGS PLACED IN VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE SOLUTION. PLANTS WITH SIMPLE LEAVES

SODIUM CHLORIDE	TIME OF OBSERVATION				
	APRIL 4 10: 30	APRIL 5 10: 00	APRIL 6 9: 00	APRIL 7 9: 00	APRIL 8 11: 00
Moles					
0.07	o	o	o	*	o
0.08	o	o	*	*	*
0.09	o	*	*	*	*
0.10	o	*	*	*	*
0.12	o	***	***	***	*
0.14	o	***	*	*d	dead
0.16	*	***	**	***	“
0.18	*	o	*d	dead	“
0.20	*	o	dead	“	“
0.22	*	o	“	“	“
0.40	*	****	“	“	“
Check	o	o	o	o	o
Check	o	o	o	o	o
Check	o	o	o	o	o

Legend: d = one leaf dead or dying
* = slightly wilted
**** = very severely wilted
** = permanently wilted
o = unwilted
*** = severely wilted

posure was sufficient to cause wilting in certain cases. Longer exposures did not usually bring about sufficient readjustment in the plant to enable it to regain turgidity after it had once wilted. The stronger solutions did not always produce wilting, although the leaves of plants in these solutions acquired a peculiar color. Such solutions may have been toxic enough to injure the limiting membranes of the absorbing cells, enabling the plant to absorb the solution of sodium chloride.

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Summary

1. The stage of wilting known as permanent wilting is not necessarily a criterion of the critical point in soil moisture usually designated as the wilting coefficient.

2. Determinations of the distribution of moisture in the different regions of the bean seedling show that a fairly large proportion of the water in the plant is to be found in the lower part of the hypocotyl. In wilting, the transpiring leaves draw upon the water in the stem as soon as the supply from the roots is cut off.

3. Plants apparently permanently wilted, which had been cut at the soil surface, revived as many as 3 or 4 times when tested in the moist chamber. The results indicate that in the stages of wilting just preceding permanent wilting the plants are using stored water from their own tissues more than they are from the soil. Therefore the critical point for soil moisture may have been reached some time previous to permanent wilting.

4. Moisture determinations on numerous bean seedlings at permanent wilting indicated that the water content at that stage in wilting is lower for plants grown at low soil moistures than for those grown in soils with higher moisture contents.

5. The mechanical tissue per unit of leaf area was found to be greater in plants grown in soil at lower moisture contents. Thus leaves from such plants have greater mechanical rigidity, and a lowered water content is not indicated so quickly by the wilting of the leaf blade.

6. Water culture plants with roots adapted to tap water were wilted by transferring them to tap water to which sodium chloride had been added to bring the concentration to an 0.08 M. solution of NaCl (3.35 atm.). A 0.07 M. solution of NaCl (2.94 atm.) very rarely produced wilting.

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METHODS OF DETERMINING GLUCOSE AND FRUCTOSE IN CORN TISSUES[1]

V. H. MORRIS AND E. F. WESP

(WITH TWO FIGURES)

Introduction

In a series of studies on the physiology of corn, considerable attention has been given to the concentration of sugars in the different tissues of the plant, especially in relation to seasonal development. In these studies the total sugar content was differentiated into free reducing sugars and sucrose. It seemed desirable to carry the investigations a step further and determine the proportions of glucose and fructose which make up the free reducing sugars. Due to the similarity in chemical properties of these two sugars, however, procedures have not been standardized for the determination of either one of them in the presence of the other and sucrose in plant tissues. It consequently was necessary to make a preliminary survey of the available methods and to work out procedures which might be satisfactory for use with samples of corn tissues.

Three of the general methods which have been used for determining glucose and fructose in mixtures of glucose, fructose, and sucrose are: (1) the oxidation of glucose in alkaline solution with iodine, (2) the oxidation of fructose with Nyns' solution, a cupro-potassium carbonate solution, and (3) the method of analyzing a mixture of three sugars by determining the combined reducing power, polarization, and the direct determination of sucrose.

Materials and methods

In our studies of corn the tissue to be sampled was prepared by passing it through a food grinder, and it was desired to develop methods which might be applicable to two kinds of sampling. In one, the "Expressed sap method," the sap was expressed from 100 gm. of the ground tissue in a laboratory hydraulic press at a pressure of 5000 lb. per sq. in. Fifty-ml. aliquots of the sap were preserved by boiling with about 150 ml. of 95 per cent. alcohol and then making up to 200 ml. when cooled to room temperature. From determinations of the weight, total solids content, and specific gravity of the sap and the total moisture content of the tissue, the quantities of sugars found in the sap were calculated to percentages of fresh tissue.

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[1] Based on investigations co-operative between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

The second kind of sample was obtained by the standard method of extracting 50 gm. of the ground tissue with 80 per cent. alcohol in a Soxhlet extractor. As the first method is quicker and simpler, the primary purpose of the extraction method was to check the accuracy of the expressed sap method.

IODOMETRIC DETERMINATION OF GLUCOSE

An excellent review of the literature and a critical study of the factors involved in the oxidation of aldose sugars, such as glucose, by iodine in alkaline solution has been reported recently by KLINE and ACREE (**4**). The reaction is usually conceived of as occurring as follows:

$$RCHO + 3NaOH + I_2 \rightarrow RCOONa + 2NaI + 2H_2O$$

Determinations of glucose were made on portions of the same cleared aliquots of the alcoholic solutions as were used for determining the reducing power by means of Fehling's solution. In the clearing, 25 ml. of the alcohol-preserved sap sample, or 50 ml. of the alcoholic extract, were pipetted into a 100-ml. volumetric flask and the alcohol driven off on a steam plate. The syrupy residue was taken up in about 50 ml. of distilled water. To this, 0.5 ml. of a saturated solution of neutral lead acetate was added. The contents of the flask were made up to volume, shaken, and filtered through a folded filter. Sufficient potassium oxalate was added to precipitate the excess lead and the solution again filtered. It was found that any of the solutions which retained some color after undergoing this clearing process contained material which was oxidized by iodine, thus introducing considerable error. Such material, however, was found to be removed by treating the solution with about 1 gm. of decolorizing carbon (Baker and Adamson, Code BA 1551 bought from General Chemical Company) and filtering again. The effect of decolorizing with carbon on the indicated glucose contents of two solutions representing leaf tissues is shown in table I. That this effect of the carbon is not due to absorption of glucose is shown by the results with a pure glucose solution given in the same table.

The oxidation of the glucose was carried out as follows: a 25-ml. aliquot of the cleared and decolorized sugar solution was pipetted into a 250-ml. iodine flask and from 5 to 10 ml. of an approximately N/10 iodine solution run in from a burette. The quantity of iodine used depended upon the quantity of glucose present, since too great an excess causes over-oxidation. Five ml. were used for samples containing up to about 40 mg. of glucose (all samples of leaf tissue), and 10 ml. for those containing more (stem, tassel, and ear samples). A 10 per cent. sodium carbonate solution was added at the rate of 1.5 ml. for each 5 ml. of iodine and the flask placed in the dark for 15 minutes. Observations indicated that the oxidation was

TABLE I

EFFECT OF DECOLORIZING CLEARED SOLUTIONS ON THE QUANTITY OF IODINE USED

SAMPLE	TREATMENT	N/10 I USED	GLUCOSE
		ml.	*per cent.*
1. Leaf tissue	Without carbon	2.90	1.97
	Carbon added	2.10	1.43
2. Leaf tissue	Without carbon	3.00	2.04
	Carbon added	2.10	1.43
3. Glucose solution	Without carbon	2.55	1.73
	Carbon added	2.55	1.73

probably complete in 5 minutes, but, since there was no evidence of increased oxidation at the end of 15 minutes, that length of time was used as being more convenient. About 2 ml. of a freshly prepared starch solution was added and the excess iodine titrated with N/20 sodium thiosulphate. About 0.5 ml. of dilute sulphuric acid was added and the iodine thus released from combination as hypoiodite or iodate was titrated with the thiosulphate. Alternate additions of the dilute sulphuric acid and the N/20 sodium thiosulphate were made until no more iodine appeared on the addition of acid. This alternate addition of small quantities of acid and thiosulphate is probably much to be preferred to the addition of a rather large excess of acid at the start of the titration in working with solutions which contain sucrose. Otherwise, hydrolysis of the sucrose by the acid might provide glucose which would be immediately oxidized by the iodine present.

The weight of glucose oxidized by the iodine is calculated on the basis that 1 ml. N/20 iodine solution = 0.0045 gm. glucose.

DETERMINATION OF FRUCTOSE BY NYNS' METHOD

The determination of fructose by the use of Nyns' solution was carried out according to the procedure described by ZERBAN and SATTLER (**8**).

Determinations were made on aliquots of the same cleared and decolorized solutions used for the determination of glucose. It was found that the decolorizing step, however, was not necessary since the same results were obtained with or without the use of carbon.

To 10 ml. of the cleared solution in a 100-ml. centrifuge tube was added 10 ml. of distilled water and 50 ml. of Nyns' solution. The mixture was kept in a constant temperature water bath at 48.5–49.0° C. for 2.5 hours. The tube was then removed and centrifuged for 5 minutes. The supernatant liquid was carefully poured off, about 15 ml. of distilled water added from a wash bottle, and the tube again centrifuged. The supernatant liquid was poured off and the copper oxide determined by dissolving in Bertrand's solution (20 per cent. sulphuric solution saturated with ferric sulphate) and titrating with N/20 potassium permanganate.

Although NYNS (5) claimed that when the factors of time, temperature, and concentration were closely guarded, the reagent showed a selective reactivity towards fructose in the presence of glucose and sucrose, this has not been substantiated by later workers, JACKSON (3), SCHUETTE and TERRILL (6), and ZERBAN and SATTLER (8). The last workers found, however, that with quantities of fructose ranging from 12 to 30 mg., and with about an equal quantity of glucose, approximately 13 mg. of glucose were required to reduce a quantity of copper equal to that reduced by 1 mg. of fructose. Preliminary determinations showed that the percentage of glucose in the free reducing sugars of corn tissues ranged from 50 to 70 per cent. The fructose-copper equivalents used in this study accordingly were determined on solutions containing 60 per cent. glucose and 40 per cent. fructose. The fructose-copper values are given in table II.

TABLE II

FRUCTOSE-COPPER EQUIVALENTS OF SOLUTIONS CONTAINING, IN ADDITION TO THE QUANTITIES OF FRUCTOSE STATED, 1.5 TIMES AS MUCH GLUCOSE

FRUCTOSE	CU
mg.	*mg.*
5	13.6
10	32.5
15	51.1
20	70.5
25	88.0
30	107.5
40	143.5

It was recognized that for proportions other than those mentioned above the quantity of copper reduced by 13 mg. of glucose might vary slightly from that reduced by 1 mg. of fructose. The sugar solutions from the samples of corn tissues, however, contained only from 2 to 18 mg. of fructose in the 10-ml. aliquots used for the fructose determination; hence, it was assumed that in solutions with such a limited range in concentration the error introduced by variations in this factor would be overshadowed by other errors.

For convenience in calculating results the chart shown as figure 1 was prepared. In this chart the abscissae represent the percentages of free reducing sugars, and the ordinates represent the milligrams of copper

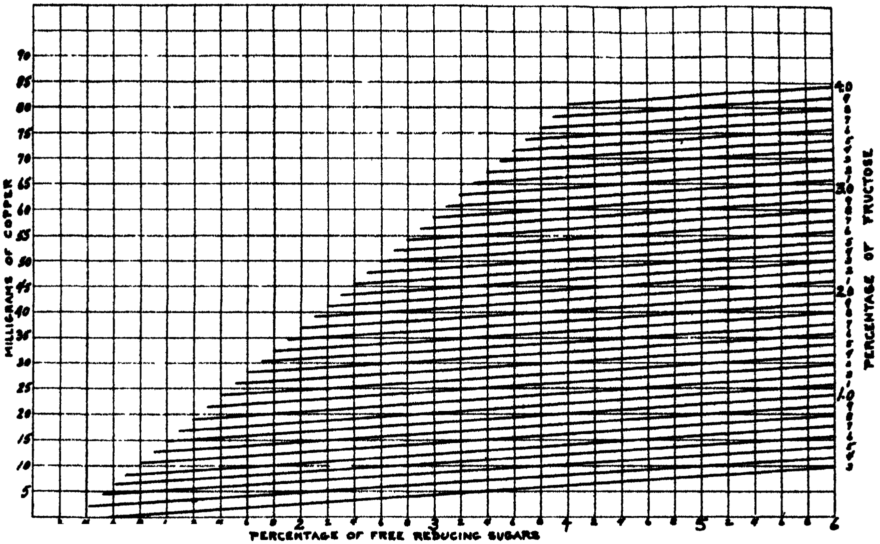

FIG. 1. Chart for calculation of percentage of fructose from determinations of percentage of free reducing sugars and milligrams of copper reduced from Nyns' solution.

reduced from Nyns' solution. By projecting the point of intersection of ordinate and abscissa for a given sample to the right hand margin, in a direction parallel to the sloping lines, the percentage of fructose may be read directly. This particular chart, of course, holds good only within the limits of the exact conditions used in this study with respect to dilution factors, etc.

POLARIZATION METHOD

A method of calculating the percentages of glucose, fructose, and sucrose in a mixture of the three sugars from determinations of reducing power, polarization, and the direct determination of sucrose has been described by BROWNE (1).

As modified for use with samples of corn tissues the calculation was based upon the following determinations: (1) the quantity of free reducing sugars by reduction of Fehling's solution; (2) the quantity of sucrose as determined by the additional reducing power after the inversion of sucrose with invertase; and (3) the polarization of the sugar solution.

The free reducing power was calculated as invert sugar and no further correction was made for the difference between the reducing ratios of glucose and fructose, since the concentrations of reducing sugars were relatively low and the range in proportions of glucose and fructose rather narrow (50 to 70 per cent. glucose).

The samples were prepared for polarization in the following manner: 100 ml. of the alcoholic expressed sap sample or 250 ml. of the alcoholic extract were transferred to a beaker and the alcohol driven off on a steam plate. The syrupy residue was taken into solution and washed into a 25-ml. volumetric flask with about 20 ml. of distilled water; 0.5 ml. of a saturated solution of neutral lead acetate then was added and the contents made up to volume and shaken. The solution was filtered through a good grade of filter-paper and the clear filtrate polarized in a 200-mm. tube, using a Schmidt-Haensch saccharimeter. The source of light was a microscope lamp containing a 400-watt stereopticon bulb.

The calculated polarization due to the sucrose present was subtracted from the observed polarization, thus giving the polarization due to the mixture of glucose and fructose. The proportions of glucose and fructose, expressed as percentage of glucose, were then obtained by use of a chart such as is illustrated in figure 2. On this chart is represented the rotation

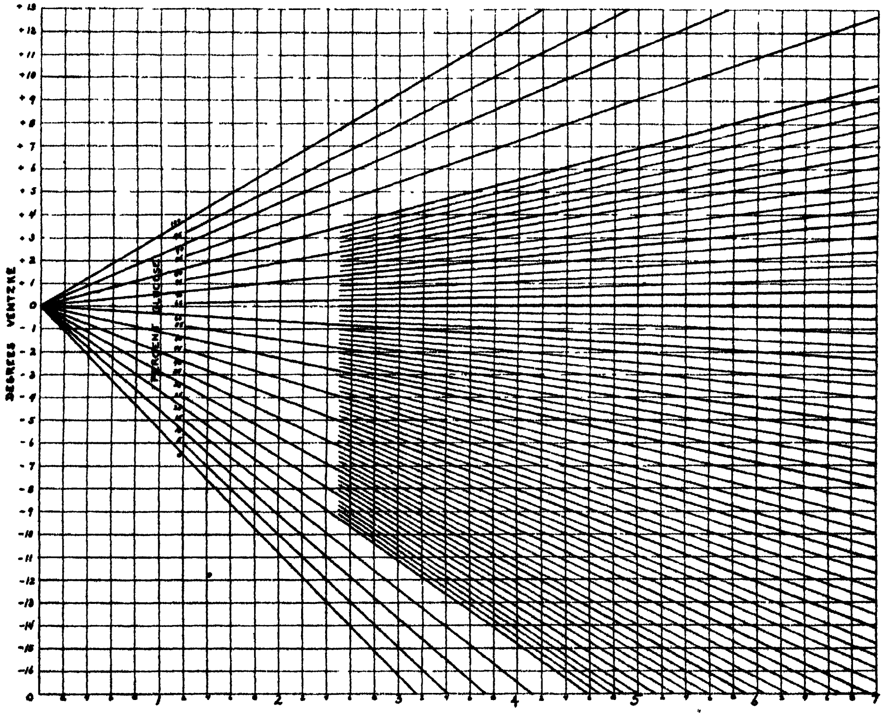

FIG. 2. Chart for calculation of percentage of glucose in free reducing sugars from determinations of free reducing sugars and their rotation.

in degrees Ventzke of all possible proportions of glucose and fructose in sugar solutions containing up to 7 per cent. free reducing sugars, calculated from their specific polarizations. On this chart the ordinates represent the rotation in degrees Ventzke due to the free reducing sugars and the abscissae represent the percentages of free reducing sugars. At the point of intersection of ordinate and abscissa for a given sample the proportion of glucose and fructose was read, expressed as percentage of glucose in the free reducing sugars.

EXAMPLE.—A sample containing 2.38 per cent. free reducing sugars and 6.02 per cent. sucrose was found to have a rotation of + 22.8° V. Since the rotation of a 6.02 per cent. sucrose solution is + 23.1° V., the 2.38 per cent. of free reducing sugars has a rotation of − 0.3° V. As shown by the chart, figure 2, 62.2 per cent. of the free reducing sugars was glucose, and therefore the sample contained 1.48 per cent. glucose and 0.90 per cent. fructose.

ANALYSIS OF PURE SUGAR SOLUTIONS

As a check on the accuracy of the three methods of determining glucose and fructose a series of 10 samples containing known quantities of glucose, fructose, and sucrose was analyzed by each of the methods. The concentrations of sugars in these samples were representative of those actually found in samples of corn tissues. The sugars were Pfanstiehl brand prepared by the Special Chemicals Co.

The samples were prepared in such a way as to simulate the preparation of the expressed sap samples previously described. The quantities of sugars for each sample were weighed out, placed in 200-ml. graduated prescription bottles, and 50 ml. of distilled water added to dissolve the sugars. The bottles were then filled nearly to the 200-ml. mark with 95 per cent. alcohol and heated in a water-bath for 30 minutes. After cooling to room temperature they were filled to the 200-ml. mark, thoroughly shaken, and set aside for analysis. In analyzing these solutions exactly the same procedures were followed as with the expressed sap samples, including the addition of neutral lead acetate and decolorizing carbon.

The composition of the samples as prepared and found by analysis is reported in table III. The determinations made were: reducing sugars before and after inversion of sucrose with invertase as measured by reduction of Fehling's solution; glucose by the iodine method; fructose by Nyns' solution; and the proportion of glucose and fructose by the polarization method, the results of the last determination being reported as glucose.

The mean quantity of glucose present in the samples was 1.71 per cent. while that indicated by the iodine method was 1.735 and by the polarimeter method was 1.728. The average quantity of fructose indicated was 1.15

TABLE III

Composition (grams per 100 ml.) of prepared solutions of pure sugars

Sample	As prepared	Composition of samples				
		As found by the method of analysis indicated				
		Glucose		Fructose	Fehling's solution	
		Iodine	Polarization	Nyns'	Free reducing sugars	Sucrose
no.		gm.	gm.	gm.	gm.	gm.
1	Glucose 0.60	0.57	0.60		1.01	
	Fructose 0.40			0.42		
	Sucrose 0.50					0.54
2	Glucose 1.20	1.20	1.20		1.97	
	Fructose 0.80			0.78		
	Sucrose 0.50					0.51
3	Glucose 1.20	1.19	1.17		1.97	
	Fructose 0.80			0.80		
	Sucrose 1.50					1.58
4	Glucose 1.50	1.58	1.52		2.45	
	Fructose 1.00			0.96		
	Sucrose 0.50					0.54
5	Glucose 1.50	1.55	1.49		2.47	
	Fructose 1.00			0.98		
	Sucrose 1.00					1.05
6	Glucose 1.50	1.54	1.49		2.47	
	Fructose 1.00			1.01		
	Sucrose 2.00					2.03
7	Glucose 1.80	1.88	1.80		2.95	
	Fructose 1.20			1.23		
	Sucrose 1.00					1.08
8	Glucose 2.40	2.38	2.55		4.06	
	Fructose 1.60			1.66		
	Sucrose 6.50					6.34
9	Glucose 2.70	2.70	2.70		4.39	
	Fructose 1.80			1.78		
	Sucrose 2.50					2.49
10	Glucose 2.70	2.76	2.76		4.47	
	Fructose 1.80			1.84		
	Sucrose 5.00					4.97
	Mean					
	Glucose 1.71	1.735	1.728			
	Fructose 1.14			1.15		

per cent. whereas the samples actually contained 1.14 per cent. This close agreement, both as to means and individual samples obtained under the conditions of the experiment, indicated that all three methods might be expected to give results well within the limits of accuracy desirable with samples of corn tissues.

COMPARISON OF METHODS WITH CORN TISSUES

The three methods were compared on corn tissues during the season of 1930. The variety of corn used was Burr-Leaming, a double cross. For convenience in sampling, the plant was divided into leaves and stem, and when the plants attained a height of approximately 120 cm. the stem was divided into upper, middle, and lower parts.

The sugar content of the samples ranged from leaf tissues containing 0.9 per cent. to stem tissues containing 5.5 per cent.

Expressed sap and alcoholic extraction samples as previously described were used. The results obtained by each of the methods were expressed as percentages of glucose on a fresh weight basis. In the case of Nyns' method the data were calculated to this basis by subtracting the quantity of fructose found from the free reducing sugars, the difference being regarded as glucose.

Single determinations were made on each of the duplicate samples taken from each lot of corn tissue. This naturally tended to make the differences between the comparable values relatively large since they reflect not only the variation due to analytic technic but also that due to sampling. The dependability of the averages representing the corn was increased by this procedure.

Results

The three methods were compared with regard to: (1) agreement in determining the mean glucose content of all samples; (2) agreement in determining the glucose content of different kinds of tissue in varying stages of development; and (3) the relative precision of the different methods in determining the glucose in duplicate samples.

EXPRESSED SAP SAMPLES

The percentages of glucose in samples of expressed sap as determined by the different methods are shown in table IV. The mean of the 30 samples was 1.767 per cent. for the iodine method, 1.780 per cent. for Nyns' method, and 1.919 per cent. for the polarization method. The significance of the differences among these means was tested, using Fisher's (**2**) formula, $t = \frac{\bar{x} - \bar{x}_1}{s}\sqrt{n}$, and the probability tables given by Student (**7**). Although the difference between means based on the iodine and Nyns' methods was only 0.013 per cent. and well might be due to chance, the odds

TABLE IV

PERCENTAGES OF GLUCOSE IN TWO KINDS OF SAMPLES OF CORN TISSUES AS DETERMINED BY THREE METHODS

DATE OF SAMPLING	TISSUE	EXPRESSED SAP SAMPLES METHOD						ALCOHOLIC EXTRACTION SAMPLES METHOD					
		IODINE		NYNS'		POLARIZATION		IODINE		NYNS'		POLARIZATION	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
July 7	Leaves	0.69	0.62	0.54	0.52	0.61	0.59	0.73	0.73	0.49	0.48	0.64	0.59
	Stems	1.26	1.23	1.24	1.17	1.21	1.19						
14	Leaves	0.91	0.86	0.69	0.68	0.71	0.71						
	Stems	1.71	1.62	1.71	1.62	1.81	1.77						
22	Leaves	0.98	0.89	0.93	0.86	1.24	1.13	0.76	0.79	0.87	0.85	1.10	1.14
	Stems	2.01	1.94	2.16	1.87	2.43	2.32	1.90	2.10	2.03	1.99	2.30	2.32
29	Leaves	0.91	0.86	1.01	0.98	0.99	0.98						
	Upper stems	2.79	2.63	3.01	2.78	2.86	2.81						
	Lower stems	2.40	2.36	2.65	2.62	2.69	2.58						
Aug. 5	Leaves	1.12	1.12	1.15	1.08	1.21	1.15	1.24	1.20	1.00	1.02	1.06	1.11
	Upper stems	2.70	2.67	2.82	2.72	3.00	2.89						
	Middle stems	2.33	2.27	2.42	2.40	2.69	2.61						
	Lower stems	2.38	2.35	2.45	2.33	2.63	2.53	2.19	2.19	2.46	2.48	2.35	2.40
11	Leaves	1.15	1.13	1.14	1.09	1.21	1.12	1.13	1.12	1.18	1.16	1.16	1.23
	Sheaths	2.28	2.28	2.30	2.18	2.41	2.39						
	Upper stems	2.59	2.54	2.78	2.77	2.99	2.93						
	Middle stems	2.54	2.49	2.67	2.65	2.82	2.81						
	Lower stems	2.46	2.27	2.41	2.33	2.58	2.56	2.30	2.30	2.50	2.64	2.55	2.79
18	Leaves	1.64	1.38	1.38	1.36	1.73	1.58	1.53	1.63	1.30	1.46	1.29	1.40
	Upper stems	2.39	2.29	2.29	2.24	2.52	2.45						
	Middle stems	2.22	2.17	2.13	2.07	2.33	2.28						
	Lower stems	2.29	2.24	2.23	2.08	2.29	2.17	2.31	2.31	2.30	2.30	2.35	2.31
25	Leaves	1.01	0.97	0.94	0.87	0.91	0.87	0.89	0.88	0.90	0.86	0.85	0.83
	Upper stems	2.21	2.18	1.88	1.85	2.10	2.07						
	Middle stems	1.85	1.82	2.06	2.03	2.28	2.28						
	Lower stems	2.12	2.03	2.23	2.17	2.36	2.34	2.00	2.00	2.40	2.28	2.49	2.56
Sept. 8	Leaves	1.05	0.97	1.15	1.06	1.26	1.17	0.96	1.14	1.00	1.00	1.16	1.18
	Upper stems	1.27	1.26	1.43	1.41	1.64	1.60						
	Middle stems	1.28	1.24	1.34	1.21	1.44	1.32						
	Lower stems	1.39	1.38	1.35	1.30	1.48	1.48	1.45	1.38	1.48	1.46	1.46	1.48
	Mean	1.767		1.780		1.919		1.506		1.534		1.619	

being 3:1, the odds were infinite against random differences as large as those between the means determined by the polarization method on the one hand and those determined by the iodine method and Nyns' method on the other.

The product moment coefficients of correlation for glucose content in the different samples were: for the iodine and Nyns' methods, 0.984 ± 0.004; for the iodine and polarization methods, 0.980 ± 0.005; and for the Nyns' and polarization methods, 0.991 ± 0.002.

The standard error of a single result, $\sqrt{\frac{\Sigma d^2}{(m-1)(n-1)}}$ is used to measure the precision of the different methods. In this formula Σd^2 indicates the sum of the squared deviations of the duplicates from their mean for each of the n (= 30) samples and m is the number in the group, here the two of each pair. The standard errors were 0.060, 0.069, and 0.053 for the iodine, Nyns', and polarization methods, respectively.

It may be concluded from this comparison of the three methods as applied to expressed sap samples that the correlation is high enough to show conclusively that the methods could be used interchangeably for determining variations due to kind and stage of development of tissues; furthermore, the precision of each of the methods was satisfactory inasmuch as all the errors are less than four per cent. of the total glucose content. On the other hand, the results obtained by the polarization method were significantly higher than those by the iodine and Nyns' method, the latter two being in good agreement.

ALCOHOLIC EXTRACTION SAMPLES

The applicability of the three methods to alcoholic extraction samples was determined on 13 samples of the series. The percentages of glucose by the different methods are included in table IV.

The means for the 13 samples were: 1.506 per cent. for the iodine method, 1.534 per cent. for Nyns' method, and 1.619 per cent. for the polarization method. The odds were 20:1 and 90:1 against random differences as large as those between the means for the polarization method on the one hand and the iodine method and Nyns' method on the other. The difference between the means for the iodine and Nyns' methods was 0.028 with odds of only 2:1 against random differences this large. In the alcoholic extraction samples the product moment coefficients of correlation for the Nyns' method with the iodine and the polarization methods were both 0.986 ± 0.005. That for determinations by the polarization and iodine methods was 0.959 ± 0.015. The standard errors were 0.061, 0.051, and 0.062 for the iodine, Nyns' and polarization methods, respectively.

The same general conclusions may be drawn from this comparison of the three methods applied to alcoholic extraction samples as were arrived at with expressed sap samples; namely, that while the methods can be used interchangeably for determining variations due to kind and stage of development of corn tissues and also are satisfactory from the point of view of precision, the mean of the results obtained by the polarization method is significantly higher than those based on the iodine and Nyns' methods, the latter two being in good agreement.

COMPARISON OF ANALYSES OF EXPRESSED SAP AND ALCOHOLIC EXTRACTION SAMPLES

With both kinds of samples the polarization method gave a mean glucose content which was significantly higher than those by the iodine and Nyns' methods; all three methods agreed reasonably with respect to the variation in samples due to differences in kind of tissue or seasonal development and also with respect to the precision in determining the glucose content in duplicate samples. Since the alcoholic extraction samples were primarily to check the accuracy of the results obtained by analyzing the expressed sap and then calculating the glucose content of the original tissue, it seemed desirable to make a direct comparison of the analyses of the 13 comparable lots of tissue for which data on both kinds of samples were available.

There were no important differences between the means for any method in the two kinds of samples. Such differences as there were well might have been due to chance, the odds not exceeding 4:1 in any comparison.

The product moment coefficients of correlation for glucose content in the two kinds of samples ranged from 0.984 to 0.996. These high correlations in connection with the close agreement of mean values are sufficient evidence that expressed sap samples may be used in place of alcoholic extraction samples without error.

DETERMINATION OF FREE REDUCING SUGARS

The determinations of glucose by oxidation with iodine and of fructose with Nyns' solution appear to give reliable results. These methods then might well replace the usual determination of free reducing sugars with Fehling's solution in some investigations.

To test this possibility, the determinations of free reducing sugars with Fehling's solution in the series of 30 expressed sap samples were compared with the sum of glucose by the iodine method and fructose by Nyns' method. The data are given in table V.

The mean of the 30 samples was 3.140 per cent. as determined with Fehling's solution and 3.127 per cent. for the sum of glucose and fructose. The difference is only 0.013, and no larger than might be expected from random sampling in one out of every 4 trials.

TABLE V

FREE REDUCING SUGAR CONTENT OF EXPRESSED SAP FROM CORN TISSUE SAMPLES AS DETERMINED BY TWO METHODS

DATE OF SAMPLING	TISSUE	FEHLING'S SOLUTION	GLUCOSE, IODINE + FRUCTOSE, NYNS
		per cent.	*per cent.*
July 7	Leaves	0.920	1.045
	Stems	2.170	2.210
14	Leaves	1.135	1.335
	Stems	3.380	3.380
22	Leaves	1.525	1.565
	Stems	3.875	3.835
29	Leaves	1.560	1.450
	Upper stems	5.300	5.115
	Lower stems	4.735	4.480
Aug. 5	Leaves	1.955	1.960
	Upper stems	5.575	5.490
	Middle stems	4.590	4.480
	Lower stems	4.310	4.285
11	Leaves	1.865	1.890
	Sheaths	4.025	4.065
	Upper stems	5.220	5.010
	Middle stems	4.650	4.505
	Lower stems	3.880	3.875
18	Leaves	2.170	2.310
	Upper stems	4.125	4.200
	Middle stems	3.615	3.710
	Lower stems	3.500	3.610
25	Leaves	1.375	1.460
	Upper stems	3.255	3.585
	Middle stems	3.385	3.175
	Lower stems	3.610	3.485
Sept. 8	Leaves	1.880	1.785
	Upper stems	2.390	2.235
	Middle stems	2.030	2.015
	Lower stems	2.190	2.250
	Mean	3.140	3.127

There is a high correlation for the free reducing sugar contents as determined by the two methods ($r = 0.997$). These methods of determining total free reducing sugars accordingly are in excellent agreement in all ways.

Discussion

In the comparison of the three methods of determining glucose in the expressed sap and alcoholic extraction samples of corn tissues used in this study it was shown that the polarization method gave results which were significantly higher than those obtained by either the iodine or Nyns' method. All attempts to discover the reason for this discrepancy have failed. The most likely source of error first appeared to be that occasioned by the defecation of materials with neutral lead acetate in the 25-ml. volumetric flask. The sugar concentration of the solution presumably is increased in direct proportion to the volume occupied by this precipitate. Moreover, as would be expected, the tendency of the polarization method to give higher results was not observed in measuring the recovery of pure sugars from solutions of known concentration. On the other hand, it would be expected that this tendency toward higher values would be most marked with those solutions which contained the largest quantities of precipitable material; namely, those representing leaf tissues. The data in table I, however, do not seem to substantiate this idea. Instead, those solutions having the higher percentages of sugars, the stem samples, are the ones which show the greatest discrepancies.

Polarization has the advantage of simplicity in technique and may well serve in preliminary studies. Moreover, the error obtained in the use of this method is not large, the difference between the means by polarization and the iodine methods being 0.142 per cent. and that between polarization and Nyns' 0.139 per cent. on a fresh weight basis, differences of less than 8 per cent. of the total glucose. The largest individual difference, that of 0.445 per cent., was found between the polarization and iodine methods on August 25 in middle stem tissue, containing about 2 per cent. of glucose.

The chief disadvantages of polarization are: (1) the large quantity of extract necessary to get a sufficient concentration of sugar for a saccharimeter reading; and (2) some extracts, particularly of leaf tissues, are so highly colored after clearing that it is extremely difficult to obtain readings with the saccharimeter even with a powerful source of light.

The oxidation of glucose by iodine is undoubtedly the most sensitive and rapid method of the three compared. Since iodine is readily absorbed or reduced by many materials contained in plant extracts, however, unusual care must be taken in the clearing and decolorizing processes. Also, the quantity of iodine solution used must be carefully regulated since too great an excess causes over-oxidation and with an insufficient excess there is under-oxidation.

The method of Nyns is much less sensitive than the iodine method and the conditions under which the oxidation is carried out fit the method well to routine analysis. Furthermore, the apparatus and most of the reagents required are those also used in the determination of reducing power by Fehling's solution. The most serious objection to the method is that the reagent is not selective for fructose, but also oxidizes glucose, making the calculation quite complicated. It would seem, however, that this method will be most valuable when used with samples, such as apple tissue in which fructose is the predominating free reducing sugar. Moreover, the results obtained in this study indicate that with the relatively small quantities of sugar found in most plant tissues, and with a rather constant glucose-fructose ratio, the calculation need not be so complicated in arriving at analyses which are within the range of experimental error of such materials.

Summary

1. With pure sugar samples all three methods gave results which were well within the limit of accuracy desired for use with samples of corn tissues.

2. The iodine and Nyns' methods gave values for glucose which were in excellent agreement in every way when used with expressed sap and alcoholic extraction samples.

3. The results obtained by the polarization method with the corn samples were consistently higher, the mean values being about 8 per cent. higher. The precision of the method, however, was equal to the other two and probably has some value in preliminary studies.

4. Either expressed sap or alcoholic extraction samples could be used equally well with all three methods.

5. Free reducing sugars may be determined from the sum of glucose by iodine and fructose by Nyns' methods with the same degree of accuracy as directly by the reducing power with Fehling's solution.

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Ohio Agricultural Experiment Station,
Wooster, Ohio.

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INVESTIGATIONS OF THE HARDINESS OF PLANTS BY MEASUREMENT OF ELECTRICAL CONDUCTIVITY[1]

S. T. DEXTER, W. E. TOTTINGHAM, AND L. F. GRABER

In a preliminary report, DEXTER, TOTTINGHAM and GRABER (**3**) have described a method of estimating the hardiness of plants as applied to three varieties of alfalfa, in which exosmosis of electrolytes from suitably frozen tissue into distilled water is determined electrometrically, and the magnitude of the electrical conductivity associated with the known hardiness of the samples. They found a progressive hardening of Grimm alfalfa, a hardy variety, as the winter season approached, and little or no hardening in a very tender strain, Hairy Peruvian. Utah Common alfalfa was intermediate in response.

The principle of increased permeability and electrical conductivity of injured tissue is a familiar one in plant physiology, while the method of electrical conductance as a measure of changing electrolyte concentration has been more or less standard for many years. (See OSTERHOUT (**16**); DIXON and ATKINS (**4**); HOAGLAND and DAVIS (**9**); HIBBARD and MILLER (**6**); HOTTES and HUELSEN (**10**); PANTANELLI (**17**); MERRILL (**13**); STILES and JORGENSON (**21**).) In the papers listed above the principle has been applied to injuries resulting from freezing, from the action of various chemicals, from pathological causes, and from mechanical rupture.

In view of the fact that the chemical and physical theories of winter-hardiness of plants have been amply discussed in recent publications, and since the bibliography has been so fully covered there, the consideration of this phase of the problem will be dismissed by reference to several papers which furnish suggestive discussions and inclusive citations of the literature. MAXIMOW (**12**) has reviewed the various physical and chemical studies of the hardiness problem in which compositional or osmotic and colloidal properties of plants are shown to be associated with hardiness. He cites, as well, numerous recent investigations in which growth recovery from controlled freezing treatment is used to estimate the degree of hardiness. NEWTON (**15**), WEIMER (**22**), STEINMETZ (**20**), ROSA (**18**) and CHANDLER (**2**) present excellent discussions, full bibliographies and additional data in regard to several chemical and physical methods of investigating the hardiness of plants. It may be stated briefly that most, if not all, of the theories of winter-hardiness are built around the idea of the water-relations of the plant, and that structural, osmotic or colloidal protection from water-withdrawal, or ice-formation form the basis of explanation for individual or varietal differences in hardiness.

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[1] Contribution from the departments of Agronomy and Agricultural Chemistry, University of Wisconsin, Madison, Wis. Published with the approval of Director.

The positive recognition of hardiness in plants, and the practical estimation of the degree of injury from freezing are matters which do not seem to have been adequately considered, nor have the various studies furnished convenient means for their accomplishment. MAXIMOW (**12**) in concluding his review of the various methods previously put forth states "we may observe that, notwithstanding a series of successful attainments, the study of the internal factors conditioning hardiness has not yet established any reliable and easily determinable symptoms for use in breeding work. For this reason, refrigerating machines—are being more and more used for the acceleration of the breeding of frost resistant varieties." Thus, the recent work of STEINMETZ (**20**), WEIMER (**22**), MARTIN (**11**), STEINBAUER (**19**), BRIERLEY and HILDRETH (**1**), HILL and SALMON (**8**), HARVEY (**5**) and others shows that varieties of alfalfa, wheat, red clover, apples and other plants can be distinguished from one another on a hardiness basis when suitably frozen after a period of hardening. In these experiments, the injury to the tissue exposed to cold temperatures is estimated by the degree of growth recovery or by microscopic observation.

The work of DEXTER, TOTTINGHAM and GRABER (**3**) has indicated that injury from controlled freezing treatments may be conveniently determined by measurement of the degree of exosmosis of electrolytes and that alfalfa varieties so examined fall into the proper order of hardiness. It is true that such measurements have not yet been adequately related to the rate or degree of recovery from freezing injury, but specific determination of injury in itself is an important step in a critical study of hardiness and its related problems.

The rapidity with which the estimation of injury can be accomplished and the convenience of the precise numerical values obtained by electrical conductance, makes the application of the method to plants other than alfalfa a matter of some interest. Moreover, as suggested above, a modification of the method might well be devised in which conductivity measurements of the tissues themselves would be made, subsequent to freezing injury, since increased conductivity might be anticipated in the injured tissues. To these ends, a series of experiments has been performed, investigating some important aspects of the hardiness problem as related to several crops under various environments.

Investigations

CONDITION OF THE ROOTS IN THE FIELD IN WINTER

In order to investigate the condition of alfalfa roots in the field in winter, samples of roots were dug from the deeply frozen ground on January

10, 1930. These roots were two years old, from plats which had received but two cuttings in the summer of 1929. Twenty-gram samples of roots were prepared in the usual manner (see Dexter, *et al.* (**3**) for details) and without further freezing treatment beyond that received in the field, were exposed to exosmosis into 50 cc. of water at 25° C. for ten hours. Table I gives the results of this trial.

TABLE I

SPECIFIC CONDUCTIVITY ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACT FROM ALFALFA ROOTS FROZEN ONLY IN THE FIELD. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

VARIETY	SPECIFIC CONDUCTIVITY
Grimm	360
Utah Common	634
Hairy Peruvian	1690

These data give definite evidence of severe field injury in the case of Hairy Peruvian, considerable injury in the case of Utah Common and but very little injury in the case of Grimm alfalfa. Conductivity of checks, in the fall, on unfrozen tissue approximated 200. Field counts in the following spring indicated almost complete loss of plants in the Hairy Peruvian plats, about 50 per cent. loss in the Utah Common plats, and under 10 per cent. loss in the Grimm plats.

A similar experiment on the same date with three varieties of winter wheat indicated precisely the same condition. The magnitudes of the electrical conductivity values and hardiness were correlated with larger exosmosis from the tenderer strains. From a ten-gram sample of crowns, exosmosis for seven hours into 40 cc. of water gave specific electrical conductivities ($x\ 10^6$) of 423 for Wisconsin Pedigree no. 2 wheat, 478 for Michigan Amber wheat and 558 for Dawson wheat. These values are quite in accord with the order of hardiness of these varieties as determined by field trials at the Wisconsin Experiment Station.

EXPERIMENTS ON GRAINS GROWN IN THE GREENHOUSE

To continue the work throughout the winter, several varieties of small grains were planted in beds and in wooden flats in the greenhouse on Jan. 27, 1930. The temperature of the greenhouse varied considerably and rarely reached a temperature as low as 60° F. during the first few weeks. Preliminary studies indicated very marked hardening effects in these greenhouse plants, with much lower exosmosis and electrical conductivity from frozen wheat crowns which had been previously exposed for a few hours to temperatures approximating 35° F.

Consequently a series of experiments was run to test several varieties in respect to hardening in a more extensive way. Samples were prepared, on each date, by removing the roots and a portion of the leaves from the individual plants leaving a clean, etiolated crown about an inch long. From such material, without washing, 2.5 gm. samples were secured, placed in Pyrex tubes and frozen in an air chamber at −9° C. for 1.5 hr. After thawing at room temperature, 20 cc. of distilled water were added to each tube and exosmosis permitted to proceed at 25° C. for 10 hr. before conductivity measurements were made. Samples were first taken on March 7 from the greenhouse maintained at approximately 60° F. and subjected to the freezing-exosmosis treatment described. The house was then cooled as much as the prevailing weather would permit. It rarely reached a temperature below 50° F. although on two successive days the house was cooled to below 40° F. for a few hours. On these two cooler days, flats containing lumps of soil in which the wheat was growing were placed outside for an additional hardening treatment at a temperature of about 32° F. Samples were prepared from the greenhouse material and from the outside plats on March 15. Table II presents the data for the three hardening treatments. The first treatment represents, roughly, wheat grown for six weeks in a 60° F. house; the second, wheat with an additional week in a 45° F. house; and the third treatment, wheat with a further additional hardening of 12 or 15 hr. at a temperature approximating 32° F.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF CROWNS OF SEVERAL VARIETIES OF WHEAT AND RYE FROZEN AT −9° C. FOR 1.5 HOURS. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

VARIETY	UNFROZEN TISSUE FROM SECOND HARDENING TREATMENT (MARCH 15)	FROZEN AFTER HARDENING TREATMENT		
		FIRST (60° F.) (MARCH 7)	SECOND (45° F.) (MARCH 15)	THIRD (32° F.) (MARCH 15)
Wheat				
Wisconsin Pedigree no. 2	141	1764	920	726
Ashkoff	164	1123	987	747
Gladden	154	1362	765	774
Honor	86	1630	765	695
Marquis	305	2355		
Progress	324	2415		
Rye				
Wisconsin Pedigree no. 2	168	882		
Spring rye	303	2155		

That hardening occurred in these winter wheats as the temperature was lowered is clearly shown. However, no definite correlation of freezing injury and electrical conductivity with varietal hardiness is evident at any stage of this hardening treatment. It is very apparent from the figures given that the hardening process was by no means complete. From data obtained at a later date from field plats (see table V) this hardening treatment would appear to be insufficient to develop the characteristic varietal differences in hardiness which occur in the field under normal fall and winter environment. It is interesting to observe that, although the hardy types, Wisconsin Pedigree no. 2 wheat and Ashkoff wheat, had assumed a very recumbent type of growth, they were in an unhardened condition on March 7 and were very sensitive to freezing. The very high values for conductivity which are found with the unfrozen tissues of spring grains are of interest and are due perhaps to the very succulent type of tissue produced under greenhouse conditions.

Chloride tests on the exudates from the wheat crowns gave values varying in a manner comparable to the conductivity values, and could be used to distinguish, at least roughly, the hardened from the unhardened tissues.

FURTHER STUDIES OF THE HARDINESS OF ALFALFA IN THE AUTUMN OF 1930

On June 27, 1930, several additional varieties of alfalfa were seeded in rows and in field plats for study the following season. In the continuation of the work with alfalfa, several modifications of the procedure seemed necessary and desirable. The June seedlings were of such a size that no attempt was made to have equal numbers of plants in the twenty-gram samples from the various varieties. From the row cultures it was impossible to obtain strictly comparable samples. Poor stands in some of the rows and excessive crowding in others gave roots and plants of widely divergent sizes and development. To the contrary, samples from the field plats were comparable, and gave satisfactory checks on duplicates. In the plats used in 1929 (now three years old) winter-killing and insect-injury of the roots made sampling somewhat difficult. No attempt was made to use eight roots in samples weighing twenty grams, as in 1929 (See DEXTER *et al.* (**3**), but the samples consisted, in 1930, of twenty gm. of representative root tissue without reference to the number of roots.

Additional low temperature facilities in 1930 permitted the use of a freezing chamber (7 cu. ft.) stirred with an electric fan, and, on occasion, of a large liquid bath. Either of these could be held at the desired temperature by thermostatic controls. The tissues were permitted to thaw by immersion of the tubes in an ice-water bath. After an hour or two, water at 0° C. was added to the tubes and exosmosis was permitted to continue for a period of twenty hours at this temperature, when electrical conductivities were determined. Exosmosis is somewhat less complete in this method than in the method used in 1929 (10 hours at 25° C.) but there is obviously less opportunity for complicating fermentations. In addition, the values given, being specific conductivities at 0° C. rather than at 25° C., must be multiplied by a constant (about 1.82) if they are to be compared with the previous figures. However, among themselves they are entirely comparable.

A series of row cultures of ten hardy strains introduced by H. L. WESTOVER, of the U. S. Department of Agriculture, was examined throughout the season. These varieties might be classed as Turkestan since they were selected in various parts of that area. All of these varieties were of a more or less dormant type. Every variety showed the same hardening effect that was reported in 1929, although some reached much lower values than others. From original conductance of approximately 750 for all varieties on October 1, final values of from 286 to 466 were reached on December 5. The varieties were rather uniform in hardiness on December 5, except for two or three varieties from the Khiva district which were hardier than the rest, as anticipated by WESTOVER. Since the varieties are unnamed, no value can be gained in this paper by enumerating the many conductivities taken on the various dates throughout the fall.

Another series of hardy varieties, in field plats, were compared as shown in table III. Several tender varieties are included for comparison. The data in this table indicate wide differences in hardiness between the very dormant types, as Hardistan or Ladak, and the vegetative types such as Hairy Peruvian and Argentine. Duplicate samples from the field plats

TABLE III

SPECIFIC CONDUCTIVITIES (x 10^6, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF ALFALFA ROOTS FROZEN 4 HOURS IN A LIQUID BATH AT −10° C. THE ROOTS WERE DUG ON DEC. 10, 1930, FROM SLIGHTLY FROZEN GROUND. AN INTERVAL OF 20 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	AGE OF PLANTS	SPECIFIC CONDUCTIVITY
Canadian Variegated	Seedling 5 mos.	377
Grimm	Seedling "	384
Ladak	Seedling "	322
Hardistan	Seedling "	281
Turkestan	Seedling "	364
Cossack	Seedling "	384
Hairy Peruvian (row)	Seedling "	593
Hairy Peruvian (plat)	Three years	513
Argentine (row)	Seedling 5 mos.	546

gave good check values for conductivity whereas the row cultures are sometimes uncertain in this respect.

APPLICATION OF THE TEST TO RED CLOVER VARIETIES

On December 9, red clover roots were dug from field plats which had been planted with barley as a nurse crop the preceding spring. The samples were prepared in the usual manner, using twenty-gram samples from the thoroughly washed and surface-dried roots. These samples were placed in Pyrex tubes, frozen four hours in a liquid bath at – 10° C. and thawed at 0° C. in ice-water. Exosmosis into 50 cc. of water at 0° C. was permitted to continue for 20 hours. Table IV indicates that a difference in hardiness existed between the Wisconsin strain grown in this state for fifty years, a variety from Missouri, and one imported from Italy. These results show that this test will differentiate the hardiness of roots of these three varieties of red clover.

TABLE IV

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF CLOVER ROOTS FROZEN 4 HOURS IN A LIQUID BATH AT – 10° C. THE ROOTS WERE DUG ON DEC. 9, 1930, FROM SLIGHTLY FROZEN GROUND. AN INTERVAL OF 20 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	SPECIFIC CONDUCTIVITY
Wisconsin	730
Missouri	900
Italy	1171

APPLICATION OF THE TEST TO WINTER-WHEAT SOWN IN FIELD PLATS AT THE USUAL RATE PER ACRE

Samples were prepared in the manner previously described, from plants dug from field plats of wheat on three dates in the fall. The samples consisted of 100 gm. of crowns. These were frozen on open screens in front of an electric fan in the freezing chamber. They were thawed in beakers immersed in ice-water. After thawing, the samples were placed in flasks, to which 750 cc. of water at 0° C. were added. Exosmosis was allowed to proceed for 16 hours at 0° C. Table V presents the results of these trials. In this table it should be noted that the freezing treatment was made more severe on December 6 than on the previous dates, since pronounced hardening had by this time rendered the plants of the hardier varieties unsusceptible to the milder freezing treatment. The table indicates the same tendency that has been described before. Following the hardening process

TABLE V

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF WINTER WHEAT CROWNS FROZEN AS INDICATED. AN INTERVAL OF 16 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	FREEZING TREATMENT ON DATE SPECIFIED		
	OCT. 25	NOV. 7	DEC. 6
	– 10° C. 2 HOURS	– 10° C. 2 HOURS	– 20° C. 2 HOURS
Minhardi	725	243	338
Wisconsin Pedigree no. 2	707	309	540
Trumbull	767	527	684

which occurred with cold weather, lower values of electrical conductance are found in exudates from the frozen tissues of varieties known to be hardy than from similar frozen tissues of tenderer strains, where greater injury from freezing occurred. In this particular case, Minhardi, a very frost-resistant wheat, is shown to be hardier than Wisconsin Pedigree no. 2 (a Turkey selection) which in turn is hardier than Trumbull.

EXPERIMENTS WITH OTHER SMALL GRAINS

Several varieties of rye, oats and barley, planted in rows gave results of a very similar nature. Table VI gives an excerpt from abundant data on several dates during the fall. The table shows the considerable degree of difference of the various varieties of rye to withstand severe freezing after all varieties had been exposed to snow and cold weather in the field. The winter and spring varieties of grain were clearly differentiated by a suitable freezing treatment at –5° C., while at –10° C. both were so severely injured that the difference is much less marked. On Oct. 15, very hardy varieties of winter wheat or rye were nearly as much injured at –5° C. as at –10° C. On the contrary, the southern winter varieties had hardened in the warm fall weather to such an extent that they were not damaged to any great degree at –5° C. but were very severely hurt by the more severe freezing treatment. There seems, therefore, to be a decided tendency for the southern winter varieties to harden in milder weather than the very hardy northern strains. An unusually warm period in November brought out the further fact that, with the exception of Minhardi winter wheat, all the ten varieties examined, showed rather definite breaking of the hardened condition with the elevated temperature, and greater injury from freezing following this period.

TABLE VI

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF SMALL GRAIN CROWNS, FROZEN AS INDICATED. AN INTERVAL OF 16 HOURS WAS ALLOWED FOR EXOSMOSIS FROM A 2-GRAM SAMPLE INTO 15 CC. OF DISTILLED WATER AT 0° C.

VARIETY	DATE	FREEZING TREATMENT	SPECIFIC CONDUCTIVITY
Rye			
Wisconsin Pedigree no. 2 winter rye	Nov. 29	2 hours –20° C.	626
Abruzzi winter rye	"	"	769
Italian winter rye	"	"	898
Spring rye	"	"	1024
Oats			
Red rust-proof winter oats	Oct. 15	2 hours –5° C.	333
Wisconsin Pedigree no. 7 spring oats	"	"	665
Red rust-proof winter oats	Oct. 15	2 hours –10° C.	907
Wisconsin Pedigree no. 7 spring oats	"	"	925
Barley			
Union winter barley	Oct. 15	2 hours –5° C.	312
Wisconsin Pedigree no. 38 spring barley	"	"	475
Union winter barley	Oct. 15	2 hours –10° C.	898
Wisconsin Pedigree no. 38 spring barley	"	"	887

ROW VERSUS NATURAL FIELD SPACING

With the small grains planted in thick rows, as with the alfalfa, less satisfactory results were obtained, poorer checks of duplicate samples were secured, and in general a less reliable indication of winter-hardiness could be gained than when the plants were growing in dense, but uniform, populations under natural field conditions. Probably the crowding, uneven covering by soil, snow and surface materials, and the competition for mineral matter contribute to these discrepancies. In the case of alfalfa, the variation in the growth of individual plants in the row was extreme. It does not appear, however, that excessive growth of the seedling plants is necessarily conducive to hardiness and moreover we have clear preliminary evidence that deficiencies, either in regard to mineral nutrition or organic foods, may be restrictive in the full development of the hardened condition. Further investigations of these relations are in progress.

DETERMINATION OF INJURY OF ALFALFA ROOTS BY MEASUREMENT OF ELECTRICAL CONDUCTIVITY OF THE TISSUE ITSELF AFTER FREEZING

In that a test of injury would be useful which involved very small portions of tissue and which would not necessitate sacrifice or even severe disturbance of the mother plant, the following method was devised. One-centimeter sections were cut from various parts of three-year-old roots of Grimm, Utah Common and Hairy Peruvian alfalfa. These sections were quickly weighed, and fastened with fine wire so that they could be suspended in tubes for freezing. The tissues were frozen by suspending the tubes for four hours in a liquid bath at the desired temperature. Thawing was allowed to proceed for several hours in the water-bath at 0° C. After warming to room temperature the root sections were placed between two electrodes actuated by a spring clamp and the electrical resistance of the tissue itself measured. The reciprocal of this resistance gives the conductivity of the section, which is divided by the weight of the section to reduce all values to approximately equal cross-sections and, therefore, comparable values. Preliminary experiments on November 5 had shown that the electrical resistance of the root section decreases very considerably as a result of freezing, and may be, in general, from one-half to one-tenth of its former value. The Grimm sections showed decidedly less change in resistance and conductivity than those from Utah Common alfalfa, while Peruvian sections were still more markedly affected. It had also appeared that pieces of small roots and especially pieces taken at some distance below the crown were more tender than large pieces, especially when taken near the crown. It therefore seemed desirable to investigate the following points in a more extensive experiment.

1. The variation in hardiness of individual plants within a variety as compared with other varieties.
2. Variation in hardiness due to position of the section on the root.
3. Variation due to the size of the root.
4. Comparison of the conductivity of the tissues themselves with values for hardiness obtained by the exosmosis method.

On November 10, eight average, uniform roots and four large roots were selected from each of the three varieties. From each of the 36 roots a two-centimeter section at the crown was removed and retained to be added to the residue of the root for exosmosis. From the first four average roots (1 to 4) of each variety, two one-centimeter sections were cut, adjacent to each other at the upper end of the remaining root. From the other four average roots (5 to 8) the upper one-centimeter section was retained, then six centimeters of root removed below it and another one-centimeter section secured. The same procedure was followed using two of the larger roots (9 and 10) for adjacent sections, and two (11 and 12) for separated sections. These sections were weighed, suspended by fine wire in moistened tubes, frozen and thawed as previously described. The results are presented in table VII. From this table it is very evident that decided differences existed in the hardiness of individual plants of any given variety on November 10. It is not assumed that these values are a measure of the ultimate hardiness of the individual plants, since they would be expected to vary somewhat in rate of hardening as winter weather approached. It

TABLE VII

VARIATIONS IN ELECTRICAL CONDUCTIVITY OF SECTIONS OF ALFALFA ROOT TISSUES FROZEN 4 HOURS AT –10° C., ON NOVEMBER 10, 1930. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

ROOT	POSITION OF SECTION	CONDUCTIVITY ($\times 10^6$) DIVIDED BY WEIGHT OF SECTION		
		GRIMM	UTAH COMMON	HAIRY PERUVIAN
1	Crown	498	891	1675
	Adjacent	745	972	1755
2	Crown	365	1410	2038
	Adjacent	407	1435	2000
3	Crown	521	618	1960
	Adjacent	617	576	2000
4	Crown	1165	892	4380
	Adjacent	1365	1100	4080
5	Crown	607	1040	1815
	6 cm. below	925	1408	1935
6	Crown	342	1302	1200
	6 cm. below	967	1400	1305
7	Crown	607	1565	1192
	6 cm. below	1209	2050	1485
8	Crown	685	1350	.
	6 cm. below	1513	1423	. .
9	Crown	192	545	1105
	Adjacent	246	631	1215
10	Crown	697	776	1465
	Adjacent	823	761	1580
11	Crown	358	792	1025
	6 cm. below	598	1338	1510
12	Crown	623	1020	1242
	6 cm. below	1053	1357	1360

is apparent that at this time of year the position of the tissue on the root is of enormous importance in the case of Grimm, of some importance in the case of Utah, and of relatively little importance in the case of Hairy Peruvian roots. The crown tissue of Grimm roots is very much less affected by freezing than the tissue more deeply buried in the soil, while crown tissue of Hairy Peruvian is but slightly different from that at other points on the root. It would appear that the crown tissue of Grimm roots has become hardened by exposure to cold, while that of the tender variety is almost unchanged. The variation due to the size of root is relatively slight, although these data are insufficient to draw definite conclusions. Further data, by this method, later in the season, appear to indicate that the small Grimm roots from field plats are more severely injured by a given freezing treatment. In this test, as in the field, large roots of Utah Common and Hairy Peruvian seem to be distinctly more winter-resistant than smaller roots. Perhaps the small roots are more completely frozen in each case and it may be that the small size of these roots is but a reflection of unfavorable growing conditions which would affect hardiness adversely. It should be remarked that the winter of 1929–30 had killed most of the Hairy Peruvian in the field and that this group of roots no doubt represents the more hardy residue from natural selection. Most of the surviving roots of Hairy Peruvian were very large. The same statements are in a measure true of Utah Common, since over half of the plants in the field were killed during that winter, while the loss of Grimm plants was less than ten per cent. These facts no doubt explain the very great variation in the Grimm roots and the relatively more uniform condition of those of the tenderer varieties. Table VIII summarizes the results presented in table VII as to the range of hardiness of the crowns within each of the three varieties.

TABLE VIII

A SUMMARY OF TABLE VII. RANGE AND AVERAGE OF CONDUCTIVITIES OF ALFALFA CROWN TISSUES AFTER FREEZING 4 HOURS AT –10° C., NOVEMBER 10, 1930

VARIETY	RANGE OF CONDUCTIVITIES OF 12 FROZEN CROWN SECTIONS	AVERAGE OF 12 CROWNS	AVERAGE OF 4 LARGE CROWNS
Grimm	192 to 1165	555	468
Utah Common	545 to 1565	1017	783
Hairy Peruvian	1025 to 4380	1736	1209

Three grams of the residue of each root were frozen and exosmosis allowed to proceed for 20 hours into 15 cc. of water in the usual way. In every case, outstandingly low values of conductivity of the exudate corresponded to low values of conductivity of the tissue itself. Since residues of the roots were used, it was quite impossible to secure samples for exosmosis identical to those used for the measurement of tissue conductivity. In some cases, the sample for exosmosis had a higher position on the root than the one-centimeter sections of which the resistance was measured; in other cases the opposite was true. However, the two methods checked in a satisfactory manner, and outstandingly hardy or tender roots were in all cases indicated by both methods, in spite of the necessary discrepancies in sampling.

Hardening of isolated root sections

It was thought desirable to investigate the artificial hardening of the lower part of the root, since this point might be of great interest in connection with the applied phases of this work. On November 18, 1930, four roots of Grimm alfalfa were cut into twelve sections each, so that each third section in order of position on the root could be frozen and examined without hardening beyond that received in the field. The remaining sections were similarly divided into two groups to be kept at 0° C. for periods of one and two weeks respectively. Thus, sections 1, 4, 7 and 10 from each root were examined without hardening treatment; sections 2, 5, 8 and 11 were kept at 0° C. for one week; while the remaining sections, 3, 6, 9 and 12, were so hardened for two weeks. The sections to be hardened were cut a few millimeters longer than those used directly, so that they could be freshly cut to the proper length just before freezing, thus avoiding drying and formation of scar tissue on the ends of the sections. The sections were frozen and thawed as in the previous experiment. The conductivity values of the tissue at room temperature divided by the weight of the samples are presented in table IX. The test indicates that very positive hardening effects are obtained by keeping isolated sections at 0° C. for a week at this time of year. The effect varies with the individual root to a marked degree, some roots showing greater ability to harden than others. As a result of two weeks of exposure to a temperature of 0° C. two of the four roots withstood a freezing treatment at –12° C. better than they did one at –10° C. previous to hardening. Although the difference between the upper and lower portions of the root is still evident after two weeks of treatment, it is not as prominent as in roots fresh from the field.

In root 1, section 7 gives a peculiar value entirely out of line with any results obtained in the many samples examined. This unusual result may have been due to super-cooling without crystallization, which is relatively common at higher freezing temperatures (as at –5° C.).

An attempt was made to harden alfalfa roots dug August 27. These roots were in a condition of relatively "low organic reserve." In this case,

TABLE IX

SPECIFIC CONDUCTIVITY ($\times 10^6$) OF ONE CENTIMETER SECTIONS OF FOUR GRIMM ALFALFA ROOTS HARDENED AND FROZEN AS INDICATED. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

SECTION	TREATMENT		CONDUCTIVITY			
	HARDENED	FROZEN	ROOT 1	ROOT 2	ROOT 3	ROOT 4
1	Field only	4 hrs. –10° C.	667	560	264	362
4	"	"	943	660	313	588
7	"	"	173	693	362	747
10	"	"	1120	767	562	862
2	Additional 1 week 0° C.	4 hrs. –10° C.	529	223	163	323
5	"	"	506	339	185	455
8	"	"	508	372	183	840
11	"	"	540	387	260	483
3	2 weeks 0° C.	4 hrs. –12° C.	634	328	285	646
6	"	"	887	385	251	777
9	"	"	814	392	307	1125
12	"	"	877	438	336	1058

ten 20-gm. samples of roots of each of the three varieties (Grimm, Utah Common and Hairy Peruvian) were prepared and placed in Pyrex tubes which were immersed in water at 0° C. for varying lengths of time. With the examination made by the exosmosis method, after freezing at –10° C. for four hours, very little or no hardening appeared to have taken place in the course of a month of such treatment, with any one of the three varieties. Duplicate samples of the three varieties taken from the hardening bath, were examined on five dates throughout the period, and the last of the "hardened" samples were finally compared on September 26 with samples fresh from the field. The "hardened" tissues were found to be very slightly more tender than the field samples. The peculiarity of failure to harden at this time of year should be examined further. It is quite possible that the attainment of complete hardening is dependent upon late fall conditions, involving such factors as short day, dormant condition, organic storage, and the predominance of sugar rather than starch in the roots.

Summary

Investigation has been devoted to a method previously proposed which indicates that the degree of hardiness of plants of certain species can be determined by measuring the extent of exosmosis of electrolytes from suitably frozen tissue. The method has been applied with satisfactory results to a large group of varieties of alfalfa, to three strains of red clover and to over twenty varieties of small grains. Tissues grown under various conditions have been examined by this method and show the marked influence of environment upon the hardening process.

An alternate method for the determination of frost resistance is presented, in which the electrical conductivity of the tissue itself is determined after freezing. With three varieties of alfalfa of known hardiness, wide varietal differences are shown, as well as marked differences in hardiness of individual plants within a given variety. The upper root tissue of a hardy variety appears to harden first, and prior to winter temperatures is shown to be decidedly more resistant to freezing injury than the more deeply buried parts. But little difference in hardiness of different parts of the root is found in the case of the most tender strain. The hardening of isolated sections of root tissue taken from the field in November is shown to occur in a refrigerator at 0° C. while hardening treatment during rapid regeneration of new growth in August was not successful.

University of Wisconsin,
Madison, Wisconsin.

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GROWTH AND DEVELOPMENT AS INFLUENCED BY FRUIT AND SEED FORMATION

A. E. MURNEEK

(WITH THREE FIGURES)

Some years ago **Briggs**, **Kidd**, and **West** (3) summarized the very extensive data which had been accumulated at a much earlier time by U. **Kreisler** and his co-workers on the growth of several field crop plants, especially *Zea mays* (**14**, **15**). The data had been lying more or less dormant and neglected up to that time. When the weekly percentage increases in dry weight (growth rate) and leaf area per unit dry weight (leaf-area ratio) were plotted against time, curves were obtained from these old records which could be roughly reduced to a generalized form (fig. 1).

The similarity in the direction of the two schematic curves thus secured suggests that the growth rate (percentage increase in dry weight) is a function of the leaf area and that it is quite constant during the life cycle of the plant. There are, however, two quite noticeable discrepancies. Growth seems to be independent of the leaf area early in the development of the plant when the seedling is still drawing on the food reserves of the

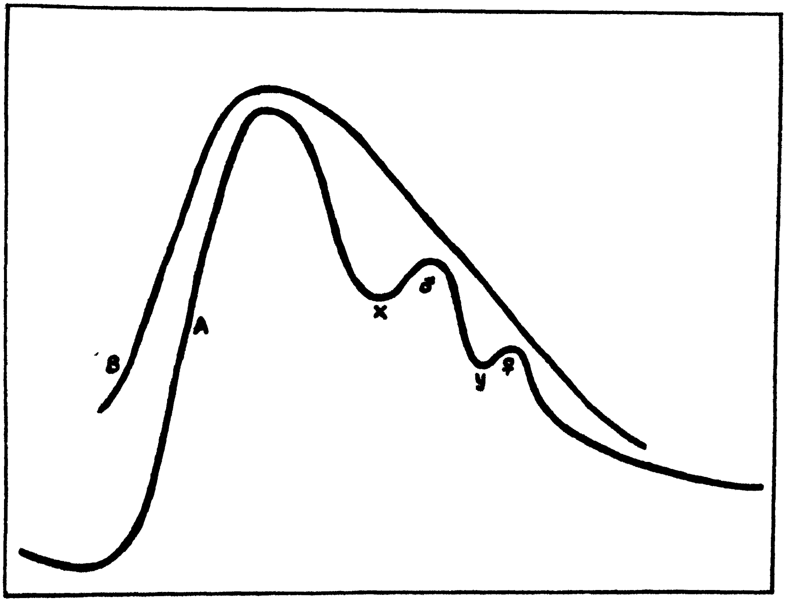

Fig. 1. Generalized growth rate (A) and leaf-area ratio (B) curves for *Zea mays*. After **Briggs**, **Kidd**, and **West** (3).

seed and while it becomes adjusted to an independent life. The second conspicuous departure of the growth curve is during the period of sexual reproduction of the plant. Since no evident connection between fluctuations in the external environmental factors and alteration in the growth rate during reproduction could be found (**14**, **15**, **3**), one is forced to conclude, as the authors were, that subsidiary minima (x, y) and maxima (♂, ♀) of the curve are due to internal causes.

It is my object to call your attention to these induced "disturbing" effects on growth and development in plants during the time of sexual reproduction. An attempt will be made to distinguish certain phases in the physiology of reproduction and to correlate these with definite stages in the morphogenesis of flowers and fruits. This discussion will be preceded and accompanied by several references to experimental data.

In his studies with the tomato (*Lycopersicon esculentum*) the writer has demonstrated (**21**, **23**) that under all types and with very extreme types of nutrition the presence of a relativity large crop of fruits results in retarded vegetative development. This was found to be particularly true, when the plants were subjected to a restricted nitrogen supply. Those on a low plane of nitrogen nutrition were completely inhibited in further growth by a single small fruit. The terminal growing point was often obliterated, the fruit becoming the "tip" of the plant as it were. But a timely removal of the fruit resulted in a complete recovery of vegetativeness and a subsequent normal growth a foot or more in length. On this new portion of the stem flowers again were produced, which, when fertilized, usually set fruit (fig. 2).

A similar situation, only on a larger scale and hence more conspicuous, was exhibited by nitrogen-high plants. These, however, required the pres-

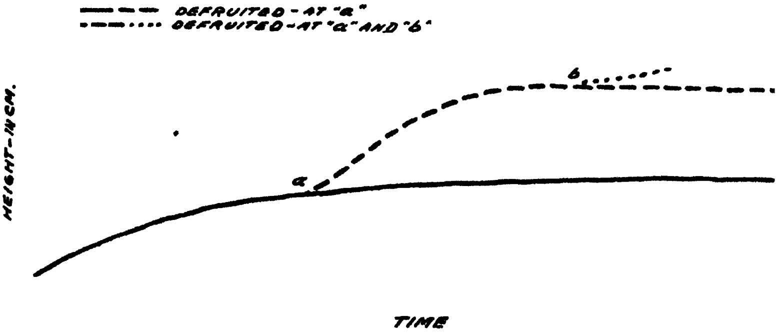

FIG. 2. Growth of normal and defruited nitrogen-low tomato plants.

ence of several large fruits—with extremely vegetative specimens as many as 30—before complete cessation of further vegetative development was brought about. When the fruits were removed from such plants a really extraordinary development took place. Frequently they attained a size twice or three times that of the control plants growing in the same containers.

It has been concluded from this work that the inhibitory effects of the fruit on development proceed in the tomato in approximately the following sequence: (a) Destruction of fecundity of the flowers. Though pollinated the blossoms could not be induced to set fruit. (b) Decrease in size of the flower clusters and all floral organs. (c) Yellowing and abortion of flower buds. (d) Decrease and cessation of further elongation of the stem. (e) Complete exhaustion and eventual death of all parts of the plant, excepting the fruit.

Continuing these investigations in further detail it was found (**24**) that tomato plants absorbed the greatest quantities of soil nutrients and synthesized the largest amounts of organic substances when fertilization (gametic union) was permitted but fruit was not allowed to develop too far. Thus fertilization and the subsequent presence of embryos exercised, during a certain period, a beneficial influence both on the vegetative parts above ground and on the root system. These conclusions are based on chemical analysis of whole plants, treated and normal specimens being grown in an identical environment. Stimulation due to sexual reproduction apparently went beyond the reproductive organs.

These results with the tomato are in complete agreement with those secured with cotton by MASON (**17, 18**), who observed a marked retardation in growth of both the central stem and lateral branches during flowering and fruiting. The rate of elongation of the main axis of the plants decreased in exact proportion to the amount of flowers formed and fruits set. When fruits were present, a conspicuous shedding of the immature flowers was also noted in the cotton. A similar observation had been made somewhat earlier by EWING (**8**), who adds that after the close of the first fruiting season, and when most of the fruits had attained a mature stage, "second growth" is developed resulting in new flowers and fruits. When flowers were removed at the time of shedding, the plants attained a much larger size and the total number of flowers was almost doubled.

Since then my attention has been called to the very interesting studies by EGOROV (**7**) and TARANOVSKY (**28**) on the effects of removal of flowers on growth and absorption of soil nutrients by various field crop plants. These publications had not come to my notice previously, because of interference in exchange in literature incident to the recent war and post-war unsettled conditions. The records gathered by these authors show that while some plants do not seem to respond greatly to defloration, the majority are affected conspicuously in growth and development. When fruits or seeds were not permitted to develop on these plants the following major physiological and morphological changes were observed: (a) A higher osmotic pressure of cell sap and probably a physical and chemical alteration in cell membranes. (b) A marked increase in development of all vegetative organs, including the roots. (c) A greater assimilation of CO_2, increased absorption of soil nutrients and higher total production of dry matter. The chemical composition of deflorated plants was naturally changed as a result of this treatment, which, for example in buckwheat, was marked by higher percentage concentration and larger total accumulation of phosphorus, potassium, magnesium, chlorine, calcium, and nitrogen. Certain differences in forms of nitrogen, calcium, and other compounds seemed to point not only to an enhanced physiological tempo and better utilization of the environment by the deflorated plants but also to other profound metabolic changes as a response to the removal of sexual organs. Unfortunately it is not stated by the authors at what particular stage of development the flowers were severed from the respective plants.

A partial corroboration of these results, especially those with legumes, may be found in several other contributions on the subject, of which the following may be mentioned: MATTIROLO, (**20**) ; HECKEL, (**10**) ; KOTOWSKY, (**13**).

The above review should make it clear that very profound physiological and chemical changes occur in all higher plants during the period of flowering and seed formation. One is thus compelled to share the opinions of several well known students of plant life (BOWER, MOLISCH, MAXIMOW) that in the potential scheme of development or the life cycle of vascular plants the most insistent and effective check seems to be that imposed by the physiological drain of flowering and fruiting.

While we do not know at present, even in a broad outline, the various physiological stages coincident with sexual reproduction, at least two major phases appear to be in evidence and have been emphasized previously by the writer (**24**). In the first place, *as a result of the act of gametic union, and hence immediately following fertilization, there is a marked increase in metabolism at the embryonic point and this increase in a measure extends throughout the body of the plant.* Secondly, *if a maximum number of embryos and their accessory tissues (seeds or fruits) under the particular conditions of nutrition are permitted to develop, the inevitable result will be a retardation in or destruction of further vegetative development, leading under extreme circumstances to death of the plant.*

An ample number of facts accumulated from defloration and other experiments can be marshaled in support of these two physiological phases of reproduction. The declining growth rate and senescence of the vegetative parts of the plant, which usually coincides with the period of embryo development, is conspicuous in many annuals. This stage in the physiology of reproduction does not seem to have received the proper emphasis.

With this consideration in mind, annual plants may be roughly grouped into those with determinate and those with indeterminate type of growth. To the first group belong practically all the cereals, grasses, etc. In these increase in size of the plant usually terminates at the time of fertilization or soon thereafter. The various vegetative structures then undergo rapid senile changes and die promptly. As this event has a strictly seasonal aspect, it thus appears that death of such plants is determined by external factors. In reality, it is due to internal causes which can be readily demonstrated by defloration experiments. Still, what is the mechanism by means of which the vegetative organs are destroyed coincident with fruiting of the plant?

An important feature during this period of development of an annual is the transfer of organic substances from the stem and foliage to the rapidly growing fruits and seeds. This, however, should have no detrimental effect on the metabolism of a plant, since the removal of the end products usually leads to a greater efficiency of physiological processes. Another very conspicuous and most significant change is the onset of yellowing of the leaves and other organs of the plant, resulting most likely from disintegration of the chloroplasts. Apparently the seeds and associated tissues draw not only on the food reserves but also on some necessary substance or substances that constitute a part of the living portion of the cell and are indispensable to the life of the chloroplasts. Thereby the very mechanism of organic synthesis is disrupted or totally destroyed and the organism perforce must die, save for the embryos and tissues closely associated with them.

Naturally, one must assume that such a condition is initiated only when the need of the reproductive organs for certain substances exceeds the current supply. The existing vegetative structures would thus be torn down to make available the necessary ingredients. Analogous instances may be found in the destruction of parental tissues by the animal embryo in case of some important metabolic deficiencies.

Considering this situation and thinking primarily of the soil nutrients which find their way into the seed, EGOROV (7) has expressed the opinion that the real cause of the senescence and death of an annual plant is the loss of magnesium by the chlorophyll. This idea seems to be based on a very abrupt kind of reasoning: Seeds absorb large amounts of the ash elements from the vegetative structures, including a comparatively high percentage of the total amount of magnesium present in the plant. And since according to WILLSTÄTTER, Mg appears to be the only mineral element present in the chlorophyll molecule, the disruption of the chloroplasts at the time of yellowing of leaves is due to this (assumed) loss of Mg. It is doubtful that this is what actually happens. The disruption of the photosynthetic apparatus does not necessarily require the direct destruction of the chlorophyll molecules. This may happen more likely as a result of the removal of large quantities of some organic substances, such as the nitrogen compounds, from the protoplasm, which is an indispensable part of the living environment for the proper functioning of chlorophyll. Neither can we accept the postulated idea that "the primary cause of the death of an annual that has flowered is the considerable loss of water at the time of flowering, which results in a decrease in assimilatory capacity of the leaves and their gradual dying" (**1**). The loss of water is not the main cause, but a subsequence of some primary influence on protoplasm in the internal physiological readjustment of a plant during sexual reproduction. In many annuals such as the cereals, where flowering lasts but a few hours, and other related processes follow in equally rapid succession, one may easily mistake and substitute one of the more conspicuous effects for the less evident cause.

There is hardly any doubt that large quantities of nitrogen are required for the development of floral structures but especially for the subsequent growth of fruits and seeds. Several investigators (**29, 11, 6, 16**) have shown that at various stages of development the grains of maize, for instance, may contain 50–70 per cent. of the total available nitrogen. Coincident with this concentration of N in the seeds a conspicuous decrease of nitrogen was found to have taken place in the vegetative parts of this plant, especially the leaves. The removal of such large quantities of nitrogen from the leaves to the seeds most likely disrupts the normal structure and function of protoplasm, and eventually leads to loss of water by the leaf and the simultaneous destruction of the chloroplasts. To be sure other indispensable elements of soil nutrition, especially phosphorus and potassium, when *in minimo,* may produce a similar effect. Under ordinary field conditions their shortage, however, is very much less likely to occur, than that of nitrogen.

From the above discussion it is apparent that an antagonism exists between vegetative growth and reproduction, which seems to be especially conspicuous in plants that flower but once, whether these are annuals (*Triticum, Zea,* etc.), biennials (*Beta, Brassica,* etc.), or perennials (*Agava, Corypha,* etc.). The last two groups of plants need not be considered in detail here, for their behavior in this respect is quite similar to the already discussed annuals. They die promptly after flowering, whether this occurs in the second year or after several years of vegetative growth, the difference being merely a matter of time.

Plants with an indeterminate type of growth, like several of our annuals (*Lycopersicon, Gossypium, Cucurbita,* some legumes, etc.) and most perennial woody plants, do not show a clear cut antagonism between vegetation and reproduction. Both functions usually proceed concurrently. The effects of the correlation between vegetative and reproductive activities, however, are expressed in a more or less clear cut manner here also. Studies with the tomato, the cotton, and certain legumes have been presented already. In all such plants, vegetative growth usually is curtailed or ceases entirely when a maximum set of fruits or seeds is obtaining. Some of the lower leaves may even be lost in this drain on the food supply by the organs of reproduction. But unless the plant becomes completely exhausted, there is, however, a recovery and resumption of vegetative development after the maturing of the first lot of fruits. Under favorable conditions of nutrition several such recurring cycles of vegetation and reproduction are commonly exhibited by such plants. Moreover, when they become large and branched the effect may become localized to a considerable degree, the fruit drawing on the available food supply primarily in that part of the plant nearest to it.

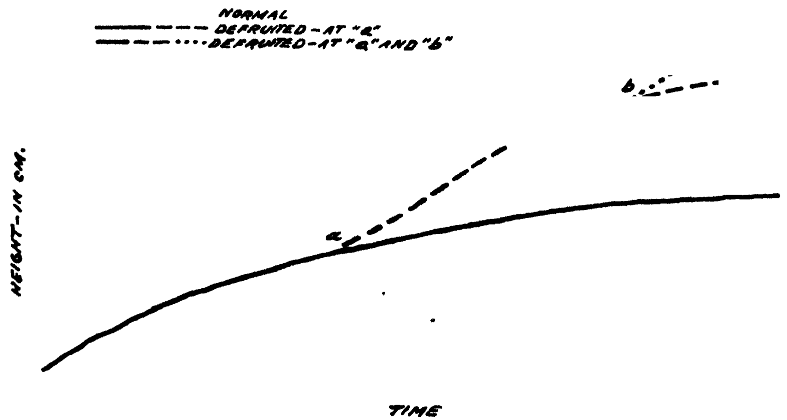

FIG. 3. Growth of normal and defruited nitrogen-high tomato plants.

The lignified perennials present a somewhat difficult situation for analysis. This complexity is due largely to the presence of relatively large amounts of storage products of diverse type and a pronounced independence of various parts of the tree. In this respect many fruit trees, for instance, should be regarded not so much as individual plants but as rather loose (metabolically speaking) aggregates of independent and competing units (**9**).

That fruiting has a dwarfing influence on the apple tree has been pointed out on several occasions (**9, 22**), but a still more pronounced effect of fruit production seems to be on its subsequent behavior. Heavily bearing trees often do not yield fruit in the following season and may require several years for a complete recovery from an excessive crop. The cyclic flowering and seed bearing of many perennials is a well known phenomenon. This behavior has a direct bearing on the fact that trees utilize for fruit production a large part of the available organic substances, particularly those containing nitrogen. After a year of heavy bearing not only is little stored, but the accumulated reserves of previous years may be heavily depleted. Nitrogen seems to play a very significant rôle in this phase of metabolism of woody plants, especially in the deciduous fruit trees; hence the emphasis on the use of nitrogen fertilizers in the orchard.

Recent studies by the writer (**22, 27**) on the nitrogen and carbohydrate distribution in organs of the bearing apple spur seem to emphasize once more the importance of nitrogen for reproduction. It was found that during flowering and fruit bearing the reproductive organs dominate the metabolism of the spur system and adjoining tissues. Flowering of the apple is characterized by a rapid increase in all active forms of nitrogen, which are moved from other parts of the tree to the flowers and subsequently to the fruits where large quantities eventually accumulate in the seeds. Concomitantly there is a corresponding decrease of N in the storage organs. It has been often observed, likewise, that heavily bearing branches of fruit trees shed their foliage much sooner than branches without or with but few fruits. Thus in this, as in many other respects, trees do not seem to differ essentially from other plants.

Another aspect of the effect of sexual reproduction on metabolism has been pointed out on several occasions and has received at least a cursory consideration. Returning to fig. 1, attention is called once more to the conspicuous departure of the growth rate curve (A) as indicated by the two subsidiary minima (x and y) which precede the time of appearance of the male and female flowers respectively. While some doubt has been expressed (**2**) whether two or only one such depression occurs, also respecting the exact time of appearance of these decreases in growth rate when considered in relationship to the period of flower development, most investigators seem to agree that they are apparent in maize and probably other cereals.

The immediate cause of this depression in accumulation of dry matter, according to some (**2, 3, 1**), is an increased respiration; while others (**2**) believe that it may result from a decreased water content of the assimilatory tissue, especially the leaves. In either case, it is supposed to be of internal origin (**3, 2**). Whatever might be the physiological processes that are instrumental in bringing about this conspicuous decrease in the growth rate, a fundamental initial cause must precede these. The writer ventures the suggestion, hypothetical as it may appear, that if such depressions in growth really occur before anthesis, then they may have some connection with the reduction division of the chromosomes preparatory to spore formation. It must be emphasized most forcibly, however, that this still uncertain effect of sexual reproduction is of an incomparably minor consequence than the aforementioned other two.

A special phase of the writer's investigations in the physiology of reproduction of plants has been an inquiry into the effects of seed development on the subsequent behavior of flowers. The disturbing influence of the presence of fruits on flower performance in the tomato has been referred to already. A favorable material for such a study is the spider flower (*Cleome spinosa*). This vigorous annual produces fruits intermittently. Sterility in this case is due primarily to the periodic abortion of pistils. By submitting a large number of plants to various experimental treatments, it was soon discovered that the developing seeds were responsible for the reduction in growth of the female reproductive organs. Undoubtedly the rapidly growing seeds were able to monopolize the organic food supply to the detriment of the flowers situated immediately above them. A timely removal of the fertilized pistils or young seed pods promptly stopped this drain and, as a consequence, all flowers developed normally and were fertile (**25, 26**).

A somewhat analogous situation was found to exist in the oil palm (*Elaeis guineensis*) (**19**). When young, this plant has two periods of flowering, one in the spring, the other in the fall. As soon as it begins to bear heavily because of age, spring flowering is reduced. This the authors explain as being due to the exhaustion of food reserves by the increased amount of fruits in the autumn, resulting in a shortage of female spadices during the spring period.

These instances tend to show rather clearly that the reproductive organs are able to exert an influence on other parts of the plant, which is somewhat varied in scope but more or less definite in character. The most important feature in the physiology of reproduction seems to be the diversion and localization by the embryos of the products of assimilation.

The question naturally arises as to what may be the means by which the developing embryos are able to draw to themselves and the adjoining tissues most of the available food supply. To accomplish such results a definite and powerful mechanism certainly must be at the disposal of the reproductive organs. At present, we are entirely ignorant as to its nature beyond the mere conjecture that it may be either of a general physiological or of a specific enzymatic or hormonic character.

In this connection one must emphasize again, though this has been done already on former occasions, that the theory proposed by CHILD (5) is worth a serious consideration. Production of gametes may be interpreted as a sign of senescence in plants, associated with a comparatively low rate of metabolism. Fertilization or gametic union, on the contrary, is the beginning of rejuvenescence, initiating high localized levels of metabolism and maintaining them throughout the time of growth of the embryo and its intimate contact with the mother sporophyte. Once a comparatively large number of such metabolic gradients are initiated, as in the case of excessive fecundity, it may lead to diverting of particular substances to the embryo or fruit and the consequent disturbances in the vegetative parts of the plant. The demand to which the sporophyte is exposed by the support of a large crop of seeds and fruits can hardly be overemphasized. Under conditions of comparatively limited absorption of soil nutrients or restricted organic synthesis, the vegetative organs of a plant may become totally exhausted due to the excessive drain by the embryos, leading eventually to death of the whole organism (**12**).

It is probable also that specific stimulating or inhibiting substances, of the nature of enzymes or hormones, may be instrumental in bringing about these effects. They may operate in the general metabolism of a plant as regulating agents. Proof has been accumulating that such catalytic or inhibitory substances do exist in the plant body. It is very likely, therefore, that they are profoundly effective in the metabolism of particular organs during certain stages of development. Such a system of secretions, being endogenous, may be initiated and may come into operation at particular stages of development of the sex organs, but especially during growth of the embryos. The consequent effect will be expressed by a specific alteration in rate or character of development of the vegetative organs or their destruction in plants that support structures devoted primarily to reproduction.

UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI.

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CHANGES ACCOMPANYING FRUIT DEVELOPMENT IN THE GARDEN PEA[1]

C. S. Bisson and H. A. Jones

(WITH FIVE FIGURES)

Introduction

Chemical studies on the garden pea were begun at the California Agricultural Experiment Station in 1924. The object of the work was to determine some of the changes that occur in the fruit during development and after harvest. This paper deals specifically with the changes in amounts of some of the components of the pods and peas during their development. The results on storage will be presented in another paper. The investigations were confined mainly to the carbohydrates as it was believed that these played a more important part than other components in the determination of quality.

Experimental procedure

The variety used was Dwarf Telephone. This is a large-podded wrinkled pea and is the variety used for the spring crop in the Imperial Valley. The seed was planted at the University Farm, Davis, California, on November 20, 1924. The plants were in full bloom on April 9, 1925, and on this date a large number of open flowers were labeled by tying strings around the peduncles. This was done to have fruit of the same age for study.

The first picking was made on April 21 when the fruit was 12 days old. The peas at this time were still very small and it was necessary to harvest a large number of fruit to get enough peas for the analyses. A second picking was made on April 27. From this date harvesting was done at two-day intervals until May 15 and then at four-day intervals until May 27. The samples were collected each morning between 7:30 and 8:00. Several representative fruits were selected at each harvest and one specimen of each picking is shown in figure 1. A number of fruits were also counted from each day's picking to be used for samples for analysis. These were then immediately shelled and the peas and pods weighed separately. From these data the average fresh weight of the fruit, pods, and peas were calculated and are given in columns 4, 5, and 7 of table I. When weighings were completed the peas and pods were preserved immediately by placing them

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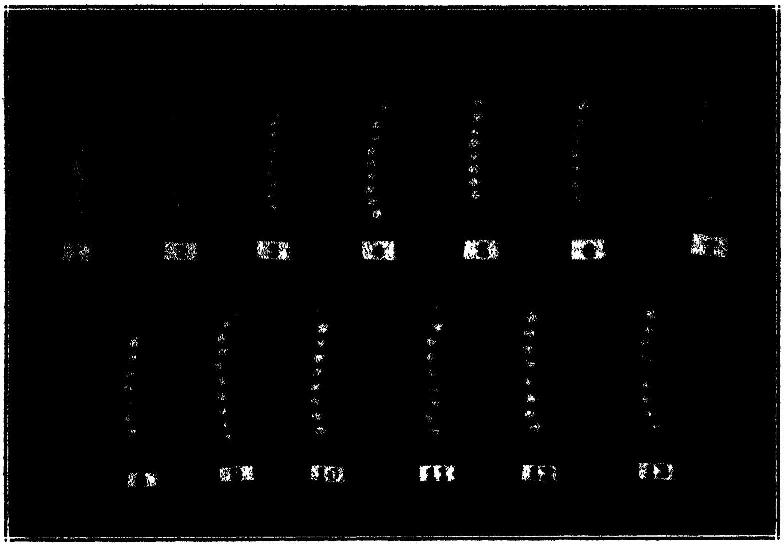


FIG. 1. Fruits at different stages of development. The table accompanying gives the ages in days and date of harvesting.

Figure number	1	2	3	4	5	6	7	8	9	10	11	12	13
Harvested. . .	4–21	4–27	4–29	5–1	5–3	5–5	5–7	5–9	5–11	5–13	5–19	5–23	5–27
Age in days	12	18	20	22	24	26	28	30	32	34	40	44	48

in separate flasks which contained sufficient 95 per cent. alcohol to produce a solution in which the alcohol content was always greater than 65 per cent. by volume after the sample had been added. To the contents of each flask there was added 0.25 gm. of $CaCO_3$; they were then heated to boiling as quickly as possible and held there for 10 minutes. After cooling, the samples were stored. Later they were analyzed for their content of dry matter, total nitrogen, reducing sugars, total sugars, starch, acid hydrolyzable components, crude fiber and ash.

Dry matter.—The stored samples were emptied into large evaporating dishes, and alcohol and water evaporated, and the residues partly dried by slowly heating over a water-bath. In order to prevent the destruction of any of the organic matter, the drying was done between 65 and 70° C. The partially dried samples were then transferred completely to small evaporating dishes and dried in air for 3 days at 65 to 68° C. Drying was completed in a vacuum oven over the same temperature range. After drying 4 days, weighings were made every 24 hours until the loss in weight for the 24-hour period became 0.2 per cent. of the dry residue. The samples

TABLE I

Growth records of fruit at different stages of growth. Variety Dwarf Telephone

1	2	3	4	5	6	7	8	9	10	11
Date harvested (1925)	Age in days	No. of fruit	Av. weight of fruit	Avegage weight of pods		Average weight of peas per pod		Av. dry weight of fruit	Fresh weight ratio pods/peas	Dry weight ratio pods/peas
				Fresh weight	Dry weight	Fresh weight	Dry weight			
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
4/21	12	150	3.33	3.02	0.37	0.31	0.05	0.42	9.7	7.0
4/27	18	110	4.90	4.04	0.59	0.86	0.14	0.73	4.7	4.3
4/29	20	113	5.11	3.66	0.57	1.45	0.26	0.83	2.52	2.2
5/1	22	116	6.13	4.22	0.61	1.91	0.35	0.96	2.22	1.8
5/3	24	122	6.31	3.98	0.59	2.33	0.47	1.06	1.71	1.3
5/5	26	108	6.65	4.06	0.57	2.59	0.55	1.12	1.57	1.1
5/7	28	101	6.60	3.82	0.54	2.78	0.66	1.20	1.37	0.81
5/9	30							....	....	....
5/11	32	86	7.22	4.00	0.52	3.22	0.82	1.34	1.24	0.64
5/13	34	117	6.21	3.18	0.41	3.03	0.93	1.34	1.05	0.44
5/15	36	118	5.31	2.44	0.36	2.87	0.93	1.29	0.85	0.39
5/19	40	105	5.59	2.42	0.36	3.17	1.09	1.45	0.76	0.33
5/23	44	106	4.80	1.66	0.37	3.14	1.22	1.59	0.53	0.31
5/27	48	139	2.15	0.45	0.36	1.70	1.17	1.53	0.26	0.30

were then ground so that they would pass completely through a 100-mesh sieve, then redried at 65 to 68° C., after which they were placed in bottles and sealed with paraffin.

The dry weights of the residues were corrected for the $CaCO_3$ added. The average dry weights of the pods and peas were calculated from the dry weight and the number of fruits in the sample. The percentages of dry matter in the pods and peas were calculated from the dry and fresh weights of the samples and they are recorded in column 3 of tables II and III. The absolute weight of each component was calculated by multiplying the average dry weight of the sample by the percentage of the component found by analysis.

Total nitrogen.—For the determination of total nitrogen duplicate samples varying from 1 to 2 gm. were used. The procedure given in SCOTT (**5**) was used in making the determinations except that phenol disulphonic acid was substituted for the salicylic acid.

Free-reducing sugars as invert sugar.—Two-gram samples were placed in Gooch crucibles and extracted for 2.5 hours in a Wiley extractor with 75 cc. of 50 per cent. (by weight) alcohol-water solution. The residues were held for starch determinations. The extracts obtained were placed in 250-cc. beakers on a steam-bath and evaporated to about 10 cc., and then completely washed into 200-cc. volumetric flasks with about 150 cc. of distilled water. In order to clarify the solution 3 to 6 cc. of a neutral saturated lead acetate solution were added to each flask; they were then thoroughly shaken and allowed to stand for 15 minutes. Water was then added to the mark; the mixtures were again shaken and then filtered through dry filter papers. Solid $Na_2C_2O_4$ was added to the filtrates in sufficient quantity to precipitate all the excess lead as PbC_2O_4. The mixtures were again filtered and 25- or 50-cc. aliquots were used for the sugar determinations by the method of MUNSON and WALKER (**4**). The Cu_2O was determined by the BISSON-SEWELL (**1**) method.

Total sugars as invert sugar.—Fifty-cc. aliquots of lead-free solutions as prepared above were pipetted into 100-cc. volumetric flasks and then 20 cc. of water and 5 cc. of concentrated hydrochloric acid added; the solutions were heated on a water-bath to 68 to 69° C. in 2.5 to 2.75 minutes, and held at this temperature for a time sufficient to make a total of 10 minutes from the instant heating was started. The solutions were then cooled at once. The solutions in the flasks were neutralized with solid Na_2CO_3 and then diluted to the graduation on the flasks with distilled water. Twenty-five or 50-cc. aliquots of these solutions were used to determine the invert sugars, as described for the determination of reducing sugars. The percentages of free reducing sugars were subtracted from the corresponding percentages of total sugar, both expressed as invert sugars and the differences multiplied by 0.95 to obtain the percentages of sucrose.

Starch.—The method for determining starch was essentially that of WALTON and COE (**6**).

Total acid hydrolyzable polysaccharides.—The method of determination was essentially the same as that used by BOSWELL (**2**). By deducting the percentage of starch the percentage of acid hydrolyzable polysaccharides other than starch was obtained.

Crude fiber.—The method used for the determination of crude fiber was that given in the official methods of analysis (**4**).

Ash.—Two two-gram samples were weighed into evaporating dishes and ashed in an electric muffle furnace at about 500° C. for 10 minutes. They were then removed from the muffle, cooled and weighed and again heated until a constant weight was obtained.

Experimental results

GROWTH OF PODS AND PEAS

After fertilization, the fruit made a fairly rapid growth. The early increase in weight of the fruit was due almost entirely to the growth of the pods, which reached their maximum weight while the peas were still very small, as shown in table I. When the fruit was 12 days old, the pods had almost reached their maximum size and by the 18th day they were about fully grown. The average *fresh weight* of the pods changed but little from the 18th to the 32nd day, and then there was a gradual decrease. The average *dry weight* of the pods showed a very large increase from the 12th to the 18th day, but little change from the 18th to the 26th day, then there was a gradual decrease until the 36th day and from that time on it remained fairly constant.

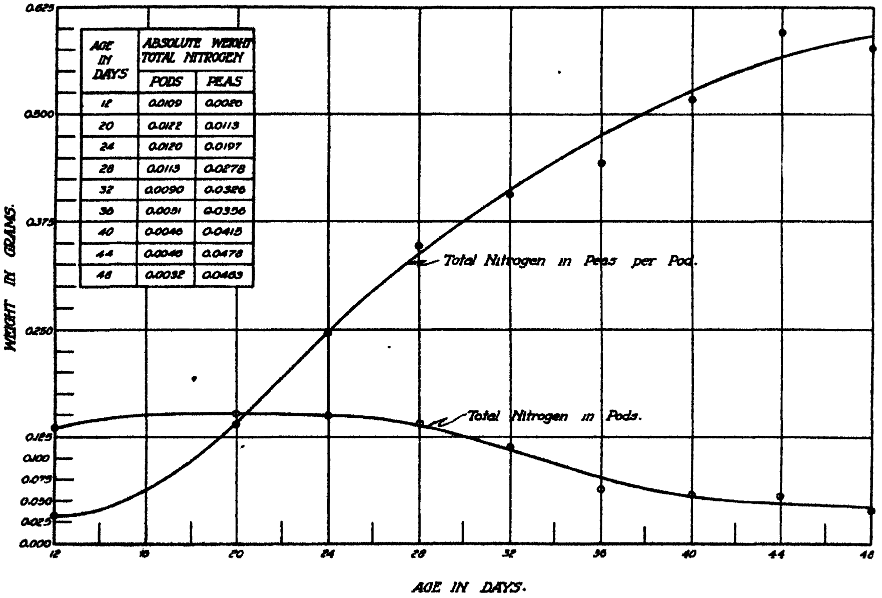
As stated above, the peas were still very small when the pods were fully developed. As shown in column 7 of table I the average *fresh weight* of peas per pod increased until about the 32nd day, then they remained fairly constant until the 44th day, after which time there was a rapid decrease due primarily to loss of moisture accompanying ripening. As shown in column 8 of table I there was a gradual increase in the average *dry weight* of peas per pod until the 44th day. This shows that even after they had reached their maximum fresh weight on the 32nd day they still continued to store reserve material until the 44th day.

As shown in table I the growth curves of the pods and peas do not coincide; the data in columns 5 and 7 show that the pods reached their maximum fresh weight about the 18th day and the peas about the 32nd day. There was a gradual increase in the average fresh weight of the entire fruit until the 32nd day. The increase from the 18th to the 32nd day must have been due entirely to the growth of the peas. There was a rather irregular decrease in the average fresh weight of the entire fruit until the 48th day. Although there was a decrease in the fresh weight over this period, the data in table I column 9 show that there was an increase in the average dry weight of fruit until the 44th day with a slight irregularity from the 32nd to the 36th day.

CHEMICAL COMPOSITION OF THE PODS

Dry matter.—The results given in column 3 of table II show that in the pods the *percentage* of dry matter remained fairly constant from the 18th to the 40th day after which time there was a rapid increase. Since the average *dry weight* remained fairly constant after the 36th day as shown in column 6 of table I the increase in the percentage of dry weight after the 40th day must have been due to the loss of water accompanying ripening.

Total nitrogen.—The *percentage* of total nitrogen as given in table II column 4 shows a general downward trend from the 12th to the 48th day. However, there were changes in the rate of decrease of percentage over certain periods of this time. From the 12th to the 20th day the decrease was very rapid, from the 20th to the 28th day there was practically no decrease, but after the 28th day there was a decrease again until the 48th day. As shown in figure 2 the *absolute weight* of total nitrogen increased



AGE IN DAYS	ABSOLUTE WEIGHT TOTAL NITROGEN	
	PODS	PEAS
12	0.0109	0.0020
20	0.0122	0.0113
24	0.0120	0.0197
28	0.0113	0.0278
32	0.0090	0.0326
36	0.0051	0.0356
40	0.0046	0.0415
44	0.0048	0.0478
48	0.0032	0.0463

FIG. 2. Absolute weight of total nitrogen in the pods and peas.

slightly from the 12th to the 20th day. It remained practically constant from the 20th to the 26th day; it decreased rapidly until the 36th day, after which time there was but a slight decrease. Since the absolute weight of total nitrogen was calculated from the percentage composition and absolute weight of dry matter, the trend of various portions of the nitrogen curve may be explained as follows: The increase in absolute weight of total nitrogen occurring from the 12th to the 20th day was primarily due to the fact that the rate of accumulation of total dry matter was greater than the rate of decrease in the percentage of nitrogen. From the 20th to the 26th day there was but a slight change in the absolute weight of dry matter and also in the percentage of nitrogen and therefore very little change in the absolute weight of total nitrogen.

Although the absolute weight of total nitrogen remained about constant between the 18th and the 26th day, this does not mean that there was no movement of nitrogen compounds into the pods but rather that the rate of inflow of nitrogen compounds was about equal to the rate of outflow. During this period there was a flow of nitrogen compounds from the stem of the plant through the pods to the peas as indicated by the rapid piling up of these substances in the peas (fig. 2). This shows that the nitrogen compounds entering the pods from the plants are not retained in the pods but are translocated to the peas. From the 26th to the 48th day there was a decrease in absolute weight of total nitrogen. This decrease may be attributed to the fact that nitrogen compounds were moving from the pods to the peas at a more rapid rate than they were entering the pods, thereby causing a drain on the reserve nitrogen compounds in the pods. The rate of drain was greatest during the early part of this period.

Total sugars.—The percentage of total sugars increased until the 24th day when the maximum was reached. From the 24th to the 30th day the percentage remained fairly constant, after which time there was a fairly rapid loss until the 48th day (table II).

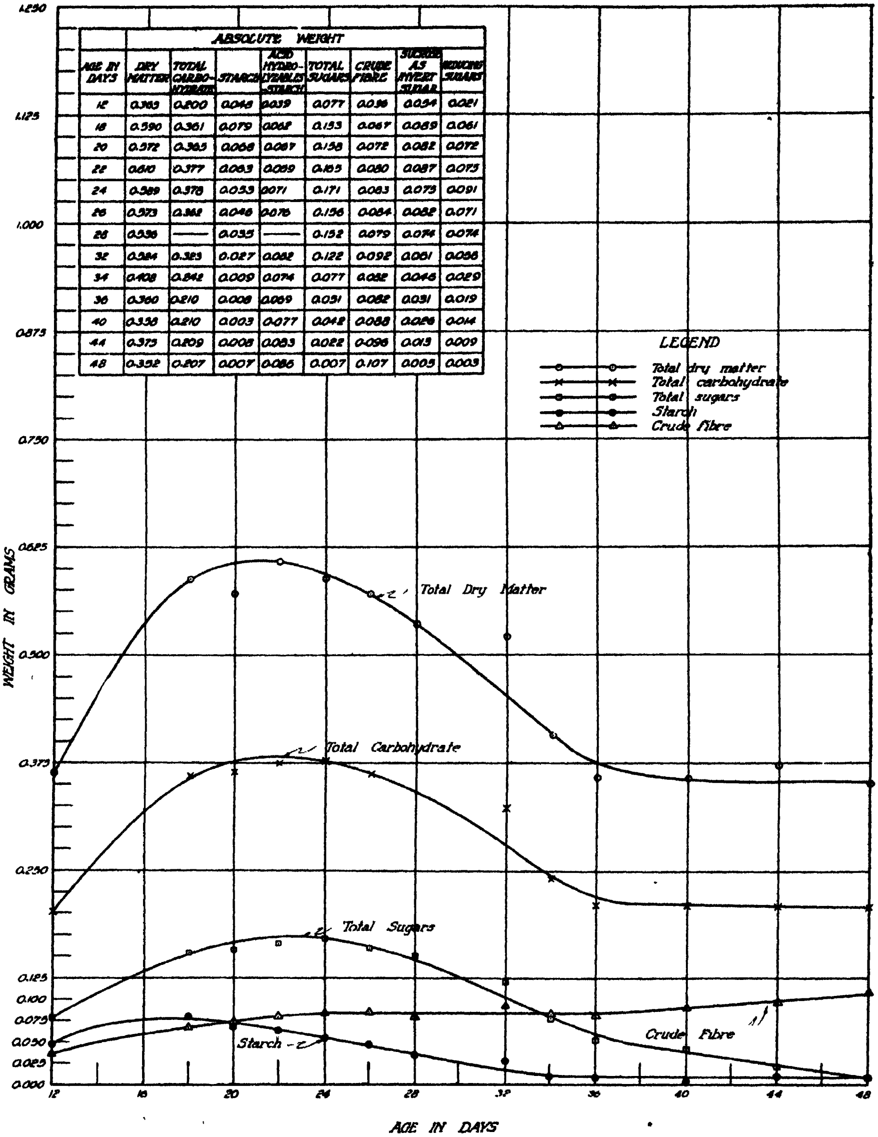
The absolute weight of total sugars also showed an increase reaching a maximum on the 24th day, after which time there was a gradual loss. The rate of loss from the 24th to the 36th day was greater than the rate of loss from the 36th to the 48th day (fig. 3). This increase in absolute weight of total sugars was probably due almost entirely to translocation to the peas rather than transformation into other classes of carbohydrates in the pods.

Starch.—It can be seen from figure 3 and table II that there was a slight increase in both the *absolute weight* and *percentage* of starch in the pods from the 12th to the 18th day, after which time there was a gradual decrease until the 34th day. The absolute weight and percentage of starch remained almost constant from the 34th to the 48th day. This showed that there was an early accumulation of starch in the pods which was prob-

TABLE II

COMPOSITION OF PODS AT DIFFERENT STAGES OF GROWTH. AMOUNTS OF CONSTITUENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

1	2	3	4	5	6	7	8	9	10	11
DATE HARVESTED (1925)	AGE	DRY MATTER IN PODS	TOTAL NITROGEN	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE	STARCH	ACID HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)	CRUDE FIBER	ASH
	days	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
4/21	12	12.11	3.00	14.77	20.93	5.85	13.02	10.72	9.92	4.51
4/27	18	14.60		15.03	25.89	10.32	13.39	10.43	11.35	.....
4/29	20	15.62	2.12	14.30	27.64	12.67	11.81	11.75	12.56	4.10
5/1	22	14.45	...	14.19	27.08	12.24	10.31	11.31	13.05	.....
5/3	24	14.79	2.05	12.75	29.02	15.46	9.05	12.06	14.04	4.32
5/5	26	14.11	.	14.27	27.28	12.35	8.07	13.18	14.66	.....
5/7	28	14.03	2.11	13.80	28.36	13.83	6.45		14.79	4.67
5/9	30	14.09	.	13.69	28.30	13.89	6.63	13.26	14.96	....
5/11	32	13.10	1.72	11.65	23.29	11.06	5.21	15.63	17.62	4.68
5/13	34	12.82	.	11.37	18.89	7.14	2.20	18.09	20.04	......
5/15	36	14.77	1.41	8.55	14.19	5.36	2.16	19.22	22.69	6.03
5/19	40	14.76	1.29	7.39	11.64	4.03	0.77	21.61	24.63	6.40
5/23	44	22.61	1.22	3.40	5.95	2.42	2.02	22.26	25.64	6.54
5/27	48	79.07	0.90	1.14	1.89	0.70	2.05	24.32	29.98	6.13



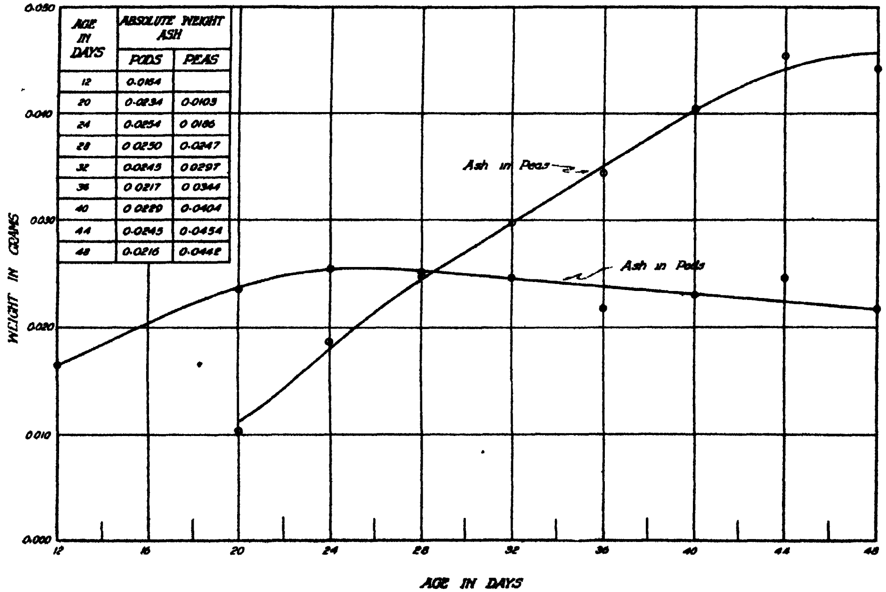
	ABSOLUTE WEIGHT							
AGE IN DAYS	DRY MATTER	TOTAL CARBO-HYDRATE	STARCH	ACID HYDRO-LYZABLES -STARCH	TOTAL SUGARS	CRUDE FIBRE	SUCROSE AS INVERT SUGAR	REDUCING SUGARS
12	0.363	0.200	0.046	0.039	0.077	0.036	0.054	0.021
18	0.590	0.361	0.079	0.062	0.153	0.067	0.089	0.061
20	0.572	0.365	0.068	0.067	0.158	0.072	0.082	0.072
22	0.610	0.377	0.063	0.069	0.165	0.080	0.087	0.075
24	0.589	0.378	0.053	0.071	0.171	0.083	0.073	0.091
26	0.573	0.362	0.046	0.076	0.156	0.084	0.082	0.071
28	0.536	——	0.035	——	0.152	0.079	0.074	0.074
32	0.524	0.323	0.027	0.062	0.122	0.092	0.061	0.058
34	0.408	0.842	0.009	0.074	0.077	0.082	0.046	0.029
36	0.360	0.210	0.008	0.069	0.051	0.082	0.031	0.019
40	0.358	0.210	0.003	0.077	0.042	0.088	0.026	0.014
44	0.375	0.209	0.005	0.083	0.022	0.096	0.013	0.009
48	0.352	0.207	0.007	0.086	0.007	0.107	0.005	0.003

FIG. 3. Absolute weight of dry matter and some of the components of the pods.

ably later hydrolyzed and translocated to the peas. From the 34th day on the starch remained fairly constant at about 2 per cent. of the total dry weight.

Acid hydrolyzable polysaccharides (minus starch).—There was an increase in the percentage of acid hydrolyzable polysaccharides (not including starch) from the 18th day to the 48th day. These compounds probably constitute a large part of the more or less permanent structure of the pod. The increase in percentage of this group of carbohydrates must be accounted for mainly by a decrease in the pods of the more mobile carbohydrates, proteins, and other compounds which move into the peas.

Crude fiber.—The percentage and absolute amount of crude fiber both show a rapid increase from the 12th to the 24th day. The increase in both the percentage and absolute weight of crude fiber during this period can be accounted for by the accumulation of crude fiber at a more rapid rate than the accumulation of total dry matter. From the 24th to the 36th day there was an increase in the *percentage* of crude fiber although not regular. Over the same period there was a decrease in the *absolute weight* of dry matter. This increase in percentage can be accounted for by the loss of constituents from the dry matter other than crude fiber. From the 36th to the 48th day there was an increase in the *percentage* of crude fiber but there was practically no change in the *absolute weight* of dry matter. This caused the absolute weight of crude fiber to show an increase over this latter period. It is possible that the building up of crude fiber during this time was due to a decrease in the rate of transfer of mobile carbohydrates to the peas and to their transformation to crude fiber in the pods.



AGE IN DAYS	ABSOLUTE WEIGHT ASH	
	PODS	PEAS
12	0.0164	
20	0.0234	0.0103
24	0.0254	0 0186
28	0 0250	0.0247
32	0.0245	0 0297
36	0 0217	0 0344
40	0 0229	0.0404
44	0.0245	0.0454
48	0.0216	0.0442

FIG. 4. Absolute weight of ash in the pods and peas.

Ash.—As shown in column 11 of table II there was a considerable decrease in the *percentage* of ash from the 12th to the 20th day. Figure 4, however, shows that there was an increase in the *absolute weight* of ash during this period. The decrease in the *percentage* of ash can be attributed to the fact that the rate of accumulation of mineral constituents in the pods was less than the rate of building up of the organic matter. If these two rates had been equal, the *percentage* of ash would have remained constant. During this period, however, there was an increase in the peas of both ash and organic matter, which showed that there was a continuous flow of these materials from the pods to the peas. From the 24th day on there appears to be a slight decrease in the absolute weight of ash. This indicates that the rate of flow of mineral constituents from the pods to the peas is slightly in excess of the flow from the remainder of the plant to the pods.

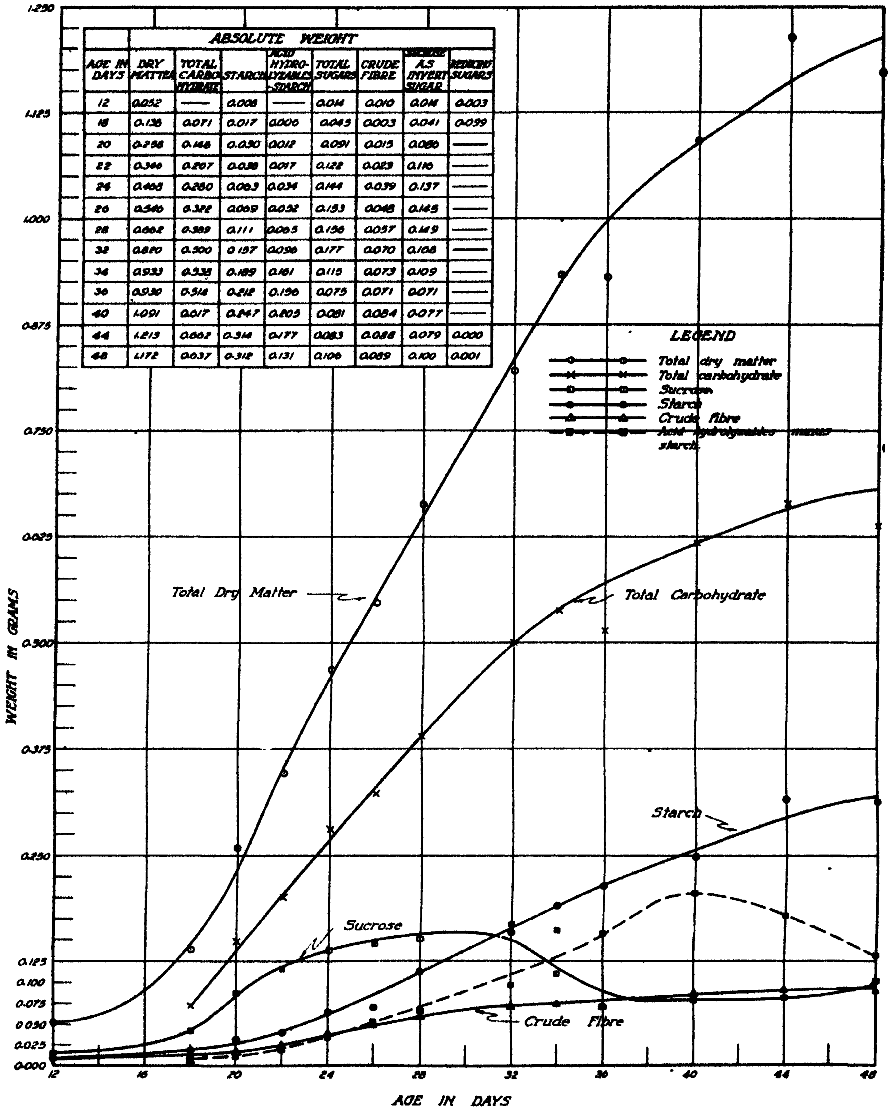
CHEMICAL COMPOSITION OF THE PEAS

Dry matter.—The percentage composition of peas at different stages of growth is given in table III. The percentage of dry matter on the 12th day was about the same as on the 18th day. After the 18th day, however, there was a regular increase until the 44th day, after which time there was a very rapid increase due to the loss of water accompanying ripening. As shown in figure 5 the rate of increase in dry weight of the peas from the 12th to the 18th day was less than the rate from the 18th to the 44th day. During the early part of this latter period the rate of increase was almost constant. The average dry weight of the peas reached a maximum at about the 44th day.

Total nitrogen.—As shown in column 4 of table III there was a gradual decrease in *percentage* of nitrogen from the 12th to the 40th day, after which time there was a slight increase. This same trend in the percentage of total nitrogen was also obtained by BOSWELL (**3**) in his studies with the variety Alaska. As shown by data in figure 2 there was a gradual increase in *absolute weight* of total nitrogen from the 12th to the 44th day. The decrease in the *percentage* of total nitrogen to the 40th day may be attributed to the fact that the rate of increase in the weight of dry matter was many times greater than the rate of increase in absolute weight of nitrogen.

Sugars.—The amounts of reducing sugars in the samples collected from the 20th to the 40th day were too small to be determined.

As shown in column 7 of table III the percentage of sucrose increased from the 12th to the 22nd day. The rate of accumulation of sucrose during this period was greater than the rate of accumulation of total dry weight, which accounts for the increase in percentage of sucrose. From the 22nd to the 44th day there was a considerable decrease in the percentage of sucrose. This may be attributed to the fact that the rate of accumulation



	ABSOLUTE WEIGHT							
AGE IN DAYS	DRY MATTER	TOTAL CARBOHYDRATE	STARCH	ACID HYDROLYZABLES -STARCH	TOTAL SUGARS	CRUDE FIBRE	SUCROSE AS INVERT SUGAR	REDUCING SUGARS
12	0.052	——	0.008	——	0.014	0.010	0.014	0.003
18	0.136	0.071	0.017	0.006	0.045	0.003	0.041	0.099
20	0.258	0.148	0.030	0.012	0.091	0.015	0.080	——
22	0.346	0.207	0.038	0.017	0.122	0.023	0.116	——
24	0.468	0.280	0.063	0.034	0.144	0.039	0.137	——
26	0.546	0.322	0.069	0.052	0.153	0.048	0.145	——
28	0.662	0.389	0.111	0.065	0.156	0.057	0.149	——
32	0.820	0.500	0.157	0.096	0.177	0.070	0.108	——
34	0.933	0.538	0.189	0.161	0.115	0.073	0.109	——
36	0.930	0.514	0.212	0.156	0.075	0.071	0.071	——
40	1.091	0.617	0.247	0.205	0.081	0.084	0.077	——
44	1.213	0.662	0.314	0.177	0.083	0.088	0.079	0.000
48	1.172	0.637	0.312	0.131	0.106	0.089	0.100	0.001

FIG. 5. Absolute weight of dry matter and some of the components of the peas.

of total dry matter together with the rate of transformation of sucrose into other carbohydrates was greater than the rate of accumulation of sucrose.

As shown in figure 5 there was a continuous increase in absolute weight of sucrose until the 32nd day; there was then a decrease until the 40th day, after which time it remained fairly constant. While the maximum *per-*

TABLE III

COMPOSITION OF SHELLED PEAS AT DIFFERENT STAGES OF GROWTH. AMOUNTS OF CONSTITUENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

1	2	3	4	5	6	7	8	9	10	11
DATE HARVESTED (1925)	AGE	DRY MATTER IN PEAS	TOTAL NITROGEN	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE	STARCH	ACID HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)	CRUDE FIBER	ASH
	days	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
4/21	12	16.66	5.16	0.53	27.74	25.84	16.33	...	1.93	....
4/27	18	15.99		0.66	33.00	30.70	12.49	4.27	2.15	....
4/29	20	17.76	4.36		35.10	33.33	11.81	4.75	5.78	3.98
5/1	22	18.17			35.34	33.57	10.97	5.04	6.77	....
5/3	24	20.09	4.22		30.71	29.17	13.43	7.33	8.40	3.89
5/5	26	21.05			28.04	26.64	12.60	9.53	8.85	....
5/7	28	23.84	4.20		23.58	22.40	16.70	9.85	8.52	3.73
5/9	30	23.50			23.86	22.67	16.06	10.87	8.37	3.26
5/11	32	25.51	3.98		21.55	20.47	19.13	11.71	8.55	3.62
5/13	34	30.77			12.28	11.67	20.22	17.25	7.78	....
5/15	36	32.40	3.83		8.07	7.67	22.83	16.79	7.66	3.70
5/19	40	34.41	3.80		7.39	7.02	22.67	18.80	7.69	3.70
5/23	44	38.71	3.93	0.04	6.86	6.48	25.84	14.55	7.31	3.74
5/27	48	68.80	3.95	0.09	9.10	8.56	26.63	11.15	7.62	3.77

centage of sucrose was reached on the 22nd day the *absolute weight* of sucrose did not reach a maximum until about the 30th day, at which time the peas were in the proper stage of development for harvest. This was about 8 days after they had reached their highest *percentage* of sucrose, but at this time the *absolute weight* of sucrose in the peas was at its maximum.

Starch.—As shown in column 8 of table III there was a decrease in *percentage* of starch from the 12th to the 22nd day, after which time there was an increase until the 48th day. The *absolute weight* of starch showed a continual increase from the 12th to about the 44th day (fig. 5).

Acid hydrolyzable polysaccharides (minus starch).—The *percentage* and *absolute weight* of acid hydrolyzable carbohydrates other than starch increased from the 18th to about the 40th day, after which time there was a decrease (table III and fig. 5).

Crude fiber.—As shown in table III there was a very rapid increase in the *percentage* of crude fiber from the 18th to about the 24th day. From about the 24th day to the 48th day the *percentage* of crude fiber remained fairly constant. There was a fairly regular increase, however, in the *absolute weight* of crude fiber from the 18th to the 48th day. The increase in the absolute weight of crude fiber during the time the peas were increasing in size can be accounted for mainly by the increase in the number of cells. After the peas had reached their maximum size the increase in crude fiber was probably due mainly to the thickening of the cell walls. After about the 24th day the *percentage* of crude fiber remained about constant but the *absolute weight* of crude fiber continued to increase. This means that the rate of increase in crude fiber was about equal to the rate of increase in total dry matter of the peas; this phenomenon would cause the percentage to remain constant.

Ash.—From the 20th to the 30th day there was a decrease in the *percentage* of ash (table III, column 11). From the 30th to the 48th day the *percentage* of ash increased slightly. The *absolute weight* of ash (fig. 4) showed a regular increase from the 20th to the 44th day. The early decrease in percentage of ash was due to the fact that the organic matter was accumulating at a more rapid rate than the ash constituents. The small increase in percentage of ash during the latter period, if significant at all, can be attributed to the fact that the rate of accumulation of ash constituents was slightly in excess of that of the organic matter.

Summary

1. The weight of the fresh fruit increased up to about the 32nd day, after which time there was a decrease due to the loss of moisture taking place at a more rapid rate than the increase in dry matter. The average

dry weight of the fruit increased throughout the entire period. The increase in dry weight of the fruit up to the 22nd day was due to the increase in weight of both pods and peas, but after the 22nd day the increase in dry weight of the fruit was due entirely to the growth of the peas.

2. In the pods there was an early maximum reached in the absolute weights of nitrogen, total sugars, starch and ash, after which there was a decline. There was a general upward trend in absolute weights of acid hydrolyzable polysaccharides other than starch and in crude fiber.

3. In the peas there was an increase in absolute weight of nitrogen, starch, crude fiber and ash throughout the entire growth period. The absolute weight of sucrose reached its maximum on about the 32nd day and then fell off rapidly. The acid hydrolyzable polysaccharides other than starch reached their maximum weight toward the latter part of the development period.

4. The crude fiber was the only constituent that showed an upward trend in absolute weight throughout the entire period in both pods and peas. The absolute weight of the acid hydrolyzable polysaccharides showed an upward trend throughout the entire period for the pods and for almost the entire period for the peas.

5. Sucrose is probably the most important constituent determining sweetness in the garden pea. In this experiment when this substance was highest in percentage the peas were still too small to be harvested. They were not at the best size for harvesting when the *percentage* of sucrose was at its maximum but they were ready for harvest at the time when the *total weight* of sucrose was at its highest.

THE UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

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SEASONAL CHANGES IN THE COMPOSITION OF THE NON-PROTEIN NITROGEN IN THE CURRENT YEAR'S SHOOTS OF BARTLETT PEAR

A. S. MULAY

(WITH SIX FIGURES)

It has been shown in a previous paper (**4**) that there are significant changes in the quantity of the non-protein nitrogen in the Bartlett pear shoots during their seasonal growth. It was interesting to know whether the quality of this nitrogen was also changed with the season. Non-protein nitrogen, therefore, was subjected to further fractionation.

Material and methods

The material used was the non-protein nitrogen filtrate, the seasonal changes in which had been studied in a previous paper (**4**). This non-protein nitrogen filtrate, obtained by extracting the samples with water and then precipitating the proteins from the extract, was fractionated before and after hydrolysis into amide, amino, basic, humin, rest, and melanin fractions by the modified VAN SLYKE method (**3**). The chart on page 116 gives a schematic picture of the complete fractionation procedure.

UNHYDROLYZED

Amide nitrogen.—This fraction was determined on an aliquot (C_2)[1] of the filtrate (C) by SACHSSE's (1873) method. Enough HCl was added to the aliquot to make a 4 per cent. HCl solution. The acidulated solution was placed in a boiling water-bath under a reflux condenser for two hours. The hydrolysate was made nearly neutral with sodium hydroxide solution and alkaline with heavy MgO. The resulting ammonia was distilled over into 0.02 N acid and titrated with 0.02 N alkali. This includes both ammonia (*i.e.*, nitrogen from ammonium salts) and amide nitrogen.

Humin nitrogen.—The alkaline mixture remaining from the amide distillation was filtered and the residue (E) washed until free of chlorides, and discarded. Combined filtrate and washings (D) were acidified with HCl, and concentrated under diminished pressure. The concentrate was diluted and aliquots (D_1) taken for nitrogen determination. Difference between the value for N.P.N. (non-protein nitrogen) (C_1) and that for amide N (C_2) plus filtrate nitrogen (D_1) gives the value for (E) humin nitrogen in the residue, *i.e.*, humin nitrogen $(E) = (C_1) - [(C_2) + (D_1)]$.

Basic nitrogen.—From the remainder of the filtrate (D) basic nitrogen was removed by phosphotungstic acid precipitation as described by MOR-

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[1] Letters in parenthesis refer to the chart.

TABLE I

NON-PROTEIN NITROGEN FRACTIONATION EXPRESSED AS PERCENTAGE OF TOTAL NON-PROTEIN NITROGEN

BARK

DATE COLLECTED	MELANIN	AMIDE		HUMIN		BASIC		AMINO		REST	
		UNHYDRO-LYZED	HYDRO-LYZED	UNHYDRO-LYZED	HYDRO-LYZED	UNHYDRO-LYZED	HYDRO-LYZED	UNHYDRO-LYZED	HYDRO-LYZED	UNHYDRO-LYZED	HYDRO-LYZED
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Sept. 16	2.3	14.8	20.5	33.4	17.5	12.3	24.8	24.2	26.0	15.2	8.9
Oct. 21	4.1	10.7	16.0	31.4	10.4	19.2	30.6	23.6	24.5	14.6	14.5
Dec. 7	3.2	13.2	16.6	20.2	8.8	25.1	31.0	16.0	24.4	25.4	15.9
Jan. 15	11.5	10.6	13.1	26.5	11.5	32.8	31.6	9.9	.	20.1	.. ..
Feb. 11	9.4	11.6	14.3	22.9	8.8	33.5	27.2	15.3		16.6	. .
Mar. 18	9.9	13.8	16.3	26.3	8.1	30.9	29.9	18.5	24.1	10.8	11.6
Apr. 18	10.3	25.4	28.2	18.9	9.3	19.2	7.9	32.8	33.0	3.6	11.3
May 13	1.0	27.4	31.4	19.2	20.4	15.4	1.0	34.3	37.4	3.4	9.4
June 24		27.0	31.2	23.0	17.7	15.1	5.4	35.9	35.9	0.4	9.9
July 15	4.7	20.6	25.8	39.0	11.4	8.7	11.6	27.6	35.8	4.1	10.6
Aug. 19	6.5	16.5	21.3	36.2	11.9	13.4	10.2	28.0	30.9	5.3	19.1
Sept. 19	4.0	13.1	18.9	32.7	10.1	20.7	21.4	25.2	29.2	8.1	16.3
Oct. 15	5.8	13.8	18.4	31.9	6.7	22.2	29.5	20.1	25.7	11.7	13.8
Nov. 17	7.5	12.3	16.7	30.7	8.6	14.4	27.4	16.2	21.9	25.5	17.7
Jan. 18	7.9	11.9	17.0	35.9	6.4	13.1	21.7	19.3	25.6	19.8	20.4

ROW (3). The filtrate (F) thus obtained was analyzed for nitrogen (F_1). The difference between the nitrogen values (D_1) and (F_1) was taken as basic nitrogen (G).

Amino nitrogen.—This fraction was determined on filtrate (F) by VAN SLYKE's method as described by MORROW. For these determinations a micro-burette was used in conjunction with a macro-deaminizing vessel of the VAN SLYKE apparatus. As the samples analyzed had no foaming tendency, caprylic alcohol was not used.

Rest nitrogen.—This fraction, also referred to in the literature as other nitrogen, was determined by the difference between nitrogen (F_1) in phosphotungstic acid filtrate (F) and amino nitrogen (F_2).

HYDROLYZED

The remainder of the filtrate (C) obtained by the removal of soluble proteins from the extract was concentrated *in vacuo* to 100 cc. and hydrolyzed with 20 per cent. HCl. The hydrolysate was filtered, the residue (I) washed until free from chlorides, and discarded. The filtrate and washings (H) were evaporated nearly to dryness under reduced pressure, and the residue was taken up with hot water and made up to volume.

Melanin nitrogen (Mel. N.), also referred to as acid insoluble humin in the literature, was determined by the difference between nitrogen value (H_1) on an aliquot of the filtrate (H) and that of N.P.N. (C_1).

Amide, humin, basic, amino, and rest nitrogen was determined as described for the unhydrolyzed sample.

Results

Tables I and II give the results of fractionation before and after hydrolysis of non-protein nitrogen of bark and wood into amide, amino, basic, humin, rest, and melanin nitrogen. The seasonal changes in the composition of non-protein nitrogen in the bark and the wood can be better seen from figures 1 and 2. Figures 3 and 4 bring out the changes resulting from hydrolysis in distribution of nitrogen among different fractions of non-protein nitrogen in bark, and figures 5 and 6 show the same for wood.

Discussion

Total non-protein nitrogen which forms about 50–80 per cent. of the total soluble nitrogen in the bark and 80–90 per cent. of it in the wood was further fractionated into amide, humin, basic, amino, and rest nitrogen before and after hydrolysis.

Amide and amino nitrogen.—The bark in 1927 shows a drop in amino nitrogen from 24 per cent. of non-protein nitrogen in September to about 10 per cent. in January (fig. 1) while the amide nitrogen keeps its level of

TABLE II

NON-PROTEIN NITROGEN FRACTIONATION EXPRESSED AS PERCENTAGE OF TOTAL NON-PROTEIN NITROGEN

WOOD

DATE COLLECTED	MELANIN	AMIDE		HUMIN		BASIC		AMINO		REST	
		UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Sept. 16	2.8	13.8	15.1	7.7	14.5	18.1	37.1	17.0	24.3	43.3	6.4
Oct. 21	1.4	5.4	8.4	12.9	12.3	34.7	50.5	9.7	13.7	40.6	13.8
Dec. 7	9.1	6.2	8.6	9.7	10.8	32.5	41.0	10.3	15.2	41.5	15.3
Jan. 15	5.6	5.4	6.3	3.0	9.7	42.9	50.5	11.2	14.7	37.4	13.2
Feb. 11	3.1	5.5	6.6	1.0	8.9	49.3	51.8	7.1	14.3	37.2	15.4
Mar. 18	5.2	5.7	6.7	2.0	8.5	51.8	55.3	8.3	9.5	32.2	14.8
Apr. 18	2.9	24.8	17.5	.3	4.6	31.3	46.4	14.8	6.3	28.6	22.4
May 13	4.1	37.0	27.9		1.4	18.9	24.3	21.8	16.7	22.6	25.4
June 24	1.6	32.4	30.7	1.8	4.4	21.0	25.1	22.7	23.9	22.0	14.2
July 15	2.5	23.0	22.2	3.0	2.0	38.1	44.8	16.1	15.2	20.2	13.4
Aug. 19	3.2	15.8	13.7	1.7	4.3	53.4	56.5	11.6	6.3	17.4	16.1
Sept. 19	7.8	12.6	9.9	2.2	1.6	59.0	58.8	8.9	7.6	17.3	14.3
Oct. 15	4.1	7.0	7.7	4.3	5.2	68.2	71.1	6.2	6.7	14.3	5.3
Nov. 17	1.3	6.2	7.7	4.6	3.1	65.7	75.4	5.8	6.5	17.7	5.9
Jan. 18	0.7	5.7	6.9	6.2	8.2	63.6	72.2	6.6	6.3	19.0	5.8

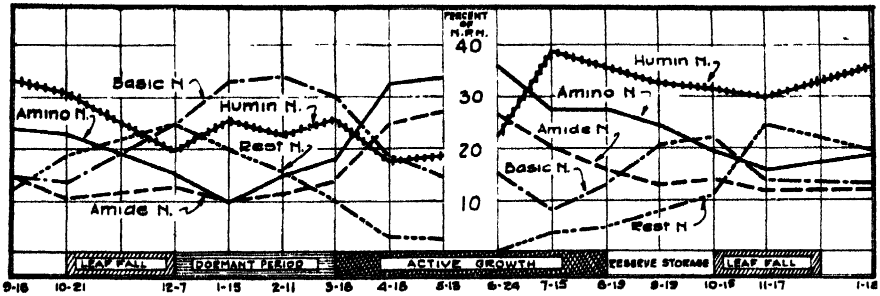


FIG. 1. Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear.

about 12 per cent. during all this time. In February both amino and amide nitrogen begin to increase and by May reach, respectively, the level of 34 and 28 per cent. of the non-protein nitrogen. The bark of 1928 shoots has high amino and amide concentration in June (36 and 27 per cent. respectively) which falls off steadily during the growing season and reaches a low level of 16 and 12 per cent., respectively, in November.

Amide and amino nitrogen in the 1927 wood (fig. 2) keep a low level of about 6 and 10 per cent., respectively, all through autumn and winter

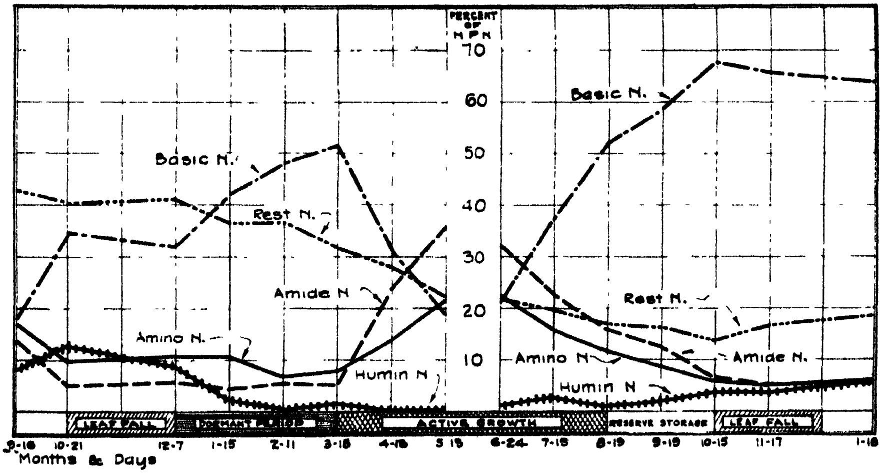


FIG. 2. Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear. (Wood, unhydrolyzed.)

and begin to rise in March. Amide nitrogen increases at a faster rate than the amino fraction and as a result by May the amide fraction is over one and one-half times as large as the amino fraction. In the 1928 shoots, the wood has about 32 per cent. amide and 23 per cent. amino nitrogen in June which falls during the growing season and reaches a low level for both of about 6 per cent. in November.

Shoot bark and wood are high in amino and amide nitrogen in May and June and decrease until they are at their lowest in winter. In the bark they begin to increase in February, sometime before the buds begin to swell, while in the wood they increase after the trees have come into full bloom. In the bark, amide nitrogen is always at a lower level than amino nitrogen while in the wood all through the active growing period amide nitrogen is decidedly higher than the amino fraction.

The general parallelism here reported between the changes in amide and amino nitrogen in both bark and wood do not agree with the results of SURE and TOTTINGHAM (6), NIGHTINGALE (5), and THOMAS (7). These workers find that the changes in amide and amino nitrogen take place in opposite directions. This may be due to differences in the material used, differences in experimental conditions, or both.

Basic nitrogen.—In the 1927 bark (fig. 1) basic nitrogen increases all through autumn and winter, attaining a maximum of about 34 per cent. in February and then falling to about 14 per cent. in May. In the 1928 bark it falls from 15 per cent. in June to 8 per cent. in July and then rises steadily to 22 per cent. in October, falls to 14 per cent. in November and remains there until January.

Basic nitrogen in the 1927 wood (fig. 2), similar to that in the bark, increases all through autumn and winter, attaining its maximum of 52 per cent. in March and then falls sharply to about 19 per cent. in May. The wood of 1928 has 22 per cent. basic nitrogen in June which rapidly increases to 68 per cent. in October and then changes but little until January.

The basic nitrogen constitutes a much higher fraction of non-protein nitrogen in the wood than in the bark. Both wood and bark start growth with a high concentration of basic nitrogen which falls in the first part of the active growth period and rises during the latter part. It continues to increase all through the dormant period. It begins to fall in the bark when the buds open and in the wood a month later. Fluctuations in basic nitrogen run in directions opposite to those in amide and amino nitrogen.

Humin nitrogen.—The humin nitrogen in the 1927 bark (fig. 1) falls from 33 to 20 per cent. of the non-protein nitrogen between September and December and remains constant the remainder of the season. In the 1928 bark it shows a sharp rise from 23 per cent. in June to 39 per cent. in July, which gradually falls to 30 per cent. in November and then rises to 36 per cent. in January.

The humin nitrogen in the 1927 wood falls from about 10 per cent. in September to about 1 per cent. in February, where it stays until May. In the 1928 wood it starts at about 1 per cent. in June and reaches about 6 per cent. by January.

Rest nitrogen.—In the 1927 bark rest nitrogen increases until December (fig. 1) when it is 23 per cent. of the non-protein nitrogen, then it steadily falls to 3 per cent. by April. The bark of 1928 has no rest nitrogen in June but it accumulates to 25 per cent. by November, after which it begins to fall.

Rest nitrogen in the 1927 wood (fig. 2) remains at 40 per cent. of the non-protein nitrogen from September to December, then falls and reaches a lower level of about 22 per cent. in May. In 1928 wood rest nitrogen shows a gradual drop from 22 per cent. in June to 14 per cent. in August and then a slow rise to 19 per cent. in January.

From the preceding discussion of the non-protein nitrogen fractions of bark and wood it appears that in June, when the first samples are taken from new growth, both bark and wood start with high amide and amino nitrogen which gradually fall, reach the lowest points after the growth in length stops, and begin to rise again when buds are swelling and opening. Basic nitrogen both in the bark and in the wood is at a low level when the first samples are taken from the new growth, reaches its maximum some time before the next year's buds begin to swell and falls sharply after the buds begin to open. In the bark, humin nitrogen increases sharply in the latter part of the growing season and then keeps at that level the remain-

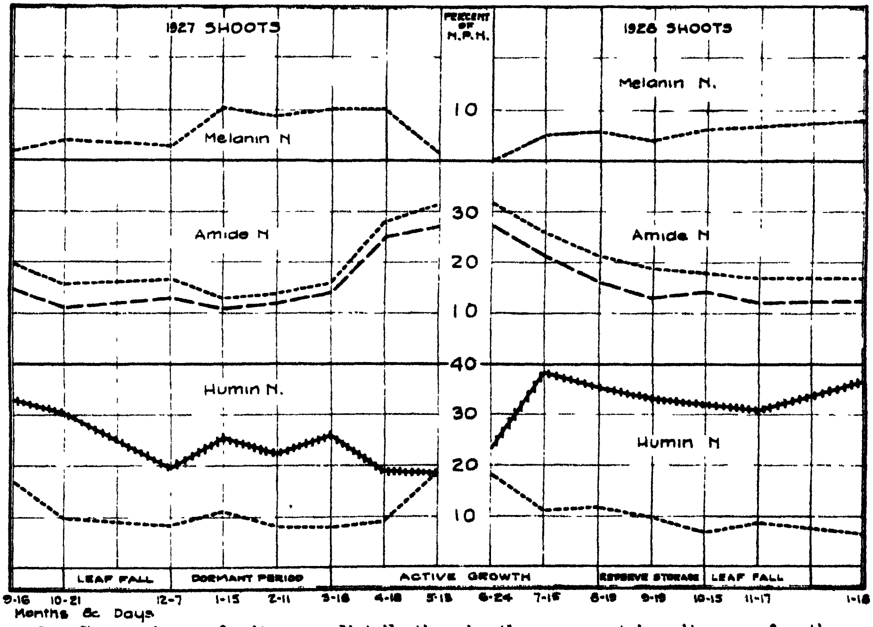


FIG. 3. Comparison of nitrogen distribution in the non-protein nitrogen fractions of bark before (solid line) and after (dotted line) hydrolysis.

der of the season, while in the wood it rises gradually after length growth stops and falls to a low level when the buds begin to swell. Rest nitrogen in the bark increases from June to December while that in the wood increases from August to December and it falls in both from January to May.

Effects of hydrolysis.—On comparing values obtained for different non-protein nitrogen fractions of bark before and after hydrolysis (figs. 3 and 4) it is seen that after hydrolysis the humin fraction diminishes and there is a concurrent increase in amino and amide nitrogen. Basic nitrogen increases in autumn and in winter when trees are dormant and decreases in spring when active growth is beginning. Rest nitrogen, on the other hand, increases during the active growing season and tends to decrease in autumn and winter. A new fraction, melanin, is formed by hydrolysis which takes its nitrogen from the amide and amino fractions. Tryptophane probably plays a most important rôle in the formation of melanin nitrogen (**2**).

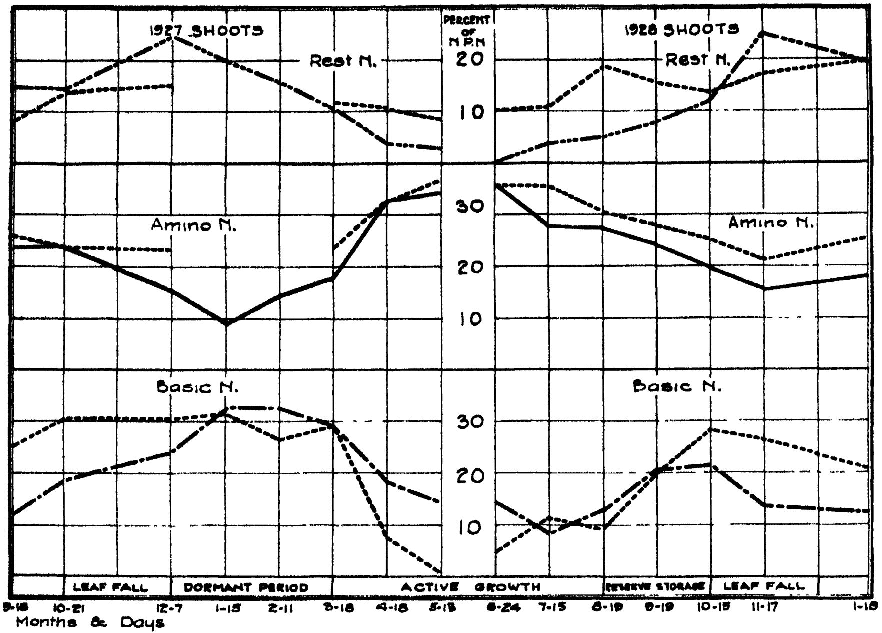


FIG. 4. Comparison of nitrogen distribution in the non-protein nitrogen fractions of bark before (solid line) and after (dotted line) hydrolysis.

The humin fraction, which is most affected by hydrolysis, seems to consist of polypeptids which on hydrolysis break up into simpler forms and are distributed into different fractions.

In the wood, rest nitrogen decreases on hydrolysis with a concurrent increase in basic nitrogen (figs. 5 and 6). This effect is most marked in the

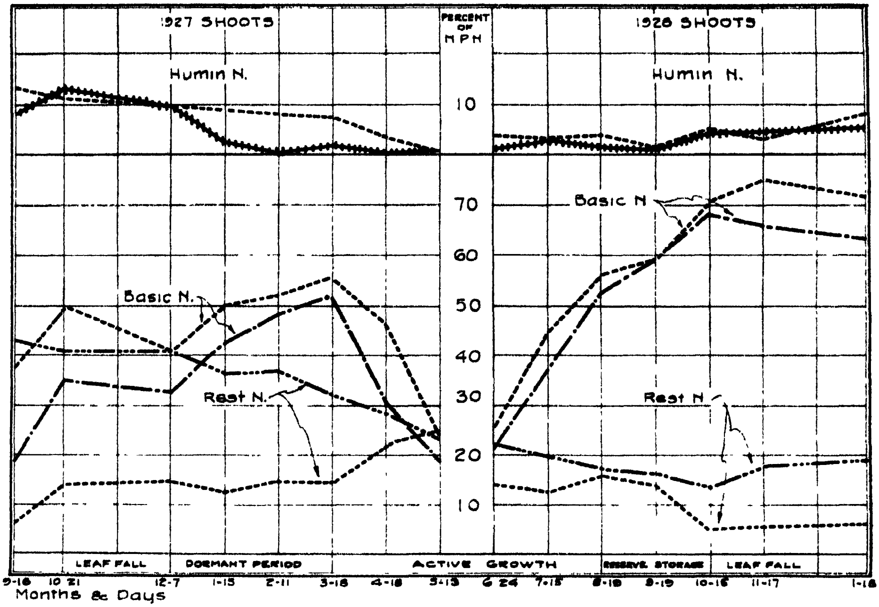


FIG. 5. Comparison of nitrogen distribution in the non-protein nitrogen fractions of wood before (solid line) and after (dotted line) hydrolysis.

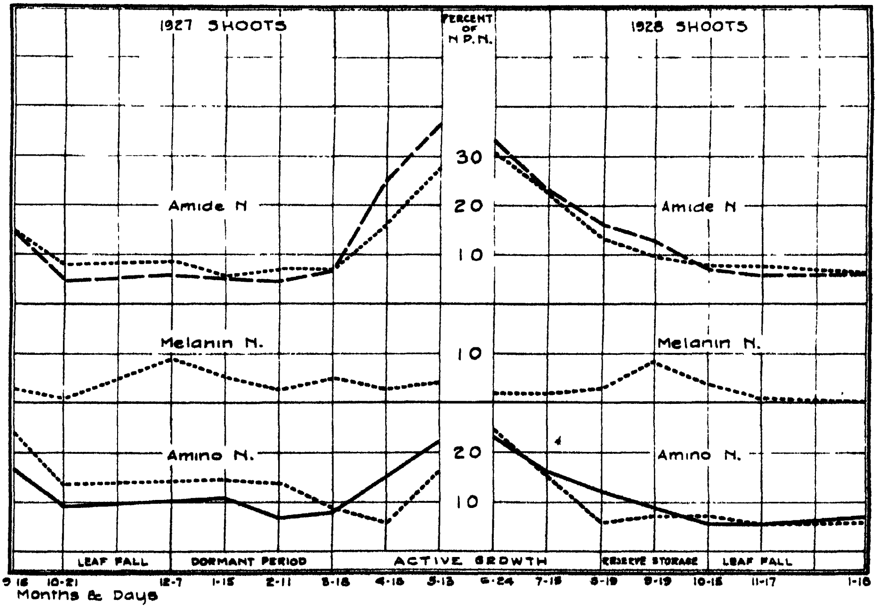
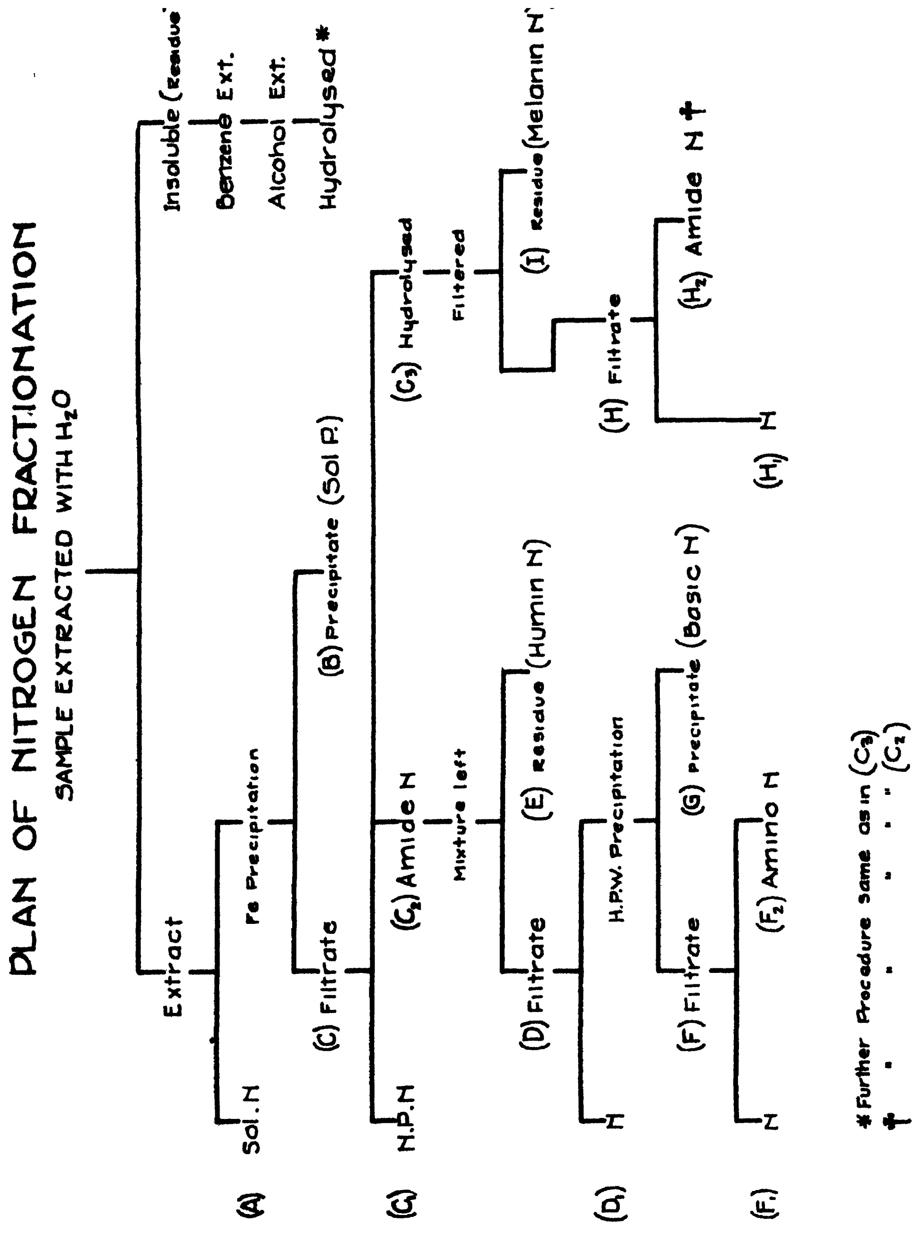


FIG. 6. Comparison of nitrogen distribution in the non-protein nitrogen fractions of wood before (solid line) and after (dotted line) hydrolysis.


PLAN OF NITROGEN FRACTIONATION
SAMPLE EXTRACTED WITH H_2O
Extract
Insoluble (Residue
Benzene Ext.
Alcohol Ext.
Hydrolysed *
(A) Sol. N
Fe Precipitation
(C) Filtrate
(B) Precipitate (Sol P.)
(C₁) N.P.N
(C₂) Amide N
(C₃) Hydrolysed
Mixture left
Filtered
(D) Filtrate
(E) Residue (Humin N)
(I) Residue (Melanin N
(D₁) N
H.P.W. Precipitation
(H) Filtrate
(F) Filtrate
(G) Precipitate (Basic N)
(H₁) N
(H₂) Amide N †
(F₁) N
(F₂) Amino N
* Further Procedure same as in (C₃)
† " " " " " (C₂)

1927 wood where basic, amino, and amide nitrogen increase at the cost of rest nitrogen. CHIBNALL (1) has found in his starvation experiments that hydrolysis decomposes the rest nitrogen compounds and suggests that this fraction may be ureide-like in nature. THOMAS (7) suggests peptid combinations, not precipitated by phosphotungstic acid, and VICKERY (8) finds that some polypeptids in alfalfa plant juice are precipitable by this reagent. Carbohydrate amino acid compounds may be suggested here as another form of hydrolyzable rest nitrogen.

Summary

The distribution of non-protein nitrogen in different fractions and their fluctuations is summarized in table III.

TABLE III

RANGE OF FLUCTUATIONS IN NON-PROTEIN NITROGEN FRACTIONS, EXPRESSED AS PERCENTAGE OF NON-PROTEIN NITROGEN

MATERIAL	AMIDE	HUMIN	BASIC	AMINO	REST
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Bark 1927	10–28	24	12–32	10–30	3–23
Wood "	6–37	0–10	20–50	10–22	20–40
Bark 1928	12–27	34	10–22	16–36	0–25
Wood "	8–32	1–6	20–65	8–23	16–20

New growth starts with high concentration of amide and amino nitrogen in both bark and wood, which falls as the growth progresses, remains at minimum in autumn and winter, and rises when new buds begin to swell. Amide nitrogen in wood is much higher than the amino nitrogen in the period of active growth. Basic nitrogen is at its lowest in June, reaches its maximum some time before new growth starts, and then falls rapidly.

On hydrolysis humin nitrogen in the bark and rest nitrogen in the wood decrease considerably, causing a concurrent increase in amide, amino, and basic nitrogen.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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CHEMICAL EQUIVALENT BASE EXCHANGE REACTIONS IN PLANTS

W. T. McGeorge

The problem of determining the causes of the movement of mineral elements through membranous tissues within the plant has elicited considerable scientific inquiry. Moreover, the factors which govern the concentration of the several ions in plant sap and of those fixed by the plant tissues have received much attention. Any observations, therefore, which relate to these phases of plant physiology and nutrition are of interest.

During recent years much work has been done on the relationships existing between the soil solution and the exchange complex of the soil, the compounds which function in the stoichiometric exchange of bases. The soil compounds associated with base exchange reactions may be divided into organic and inorganic groups. The latter have been extensively investigated, but the former have received comparatively little attention. While the absorbing power of so-called humic bodies has been recognized for many years, comparatively little study has been devoted to quantitative values and to the nature of the absorbing compounds. At the time the exchange property of the clay fraction of the soil was demonstrated the same property was assumed for the organic fraction, but later some doubt arose as to the validity of this assumption. This problem has been rather intensively studied at the Arizona Agricultural Experiment Station (**3**) and it has been demonstrated beyond question that the organic fraction of the soil possesses a chemically equivalent exchange property, and that it is a linear function of the carbon content of the organic material.

Of the carbon compounds which compose the organic fraction of the soil the pentosans, lignins, proteins and cellulose are most abundant. Of these, lignin appeared most likely to possess base exchange properties and it was therefore chosen for study. While the chemical composition of lignin has not been definitely established, it is known to possess phenolic hydroxyl groups through which basic lignates may be formed. Lignin as isolated from a number of soils and from plant materials was studied and all samples were found to possess the property of exchanging one base for another in chemically equivalent proportions. On quantitatively determining the percentage of lignin and the replacement capacity of highly organic soils, a linear relationship between them was noted. The base-absorbing capacity of lignin varied with the source, and with the method used in preparing it from the mother substance. For example, lignin extracted from soils and plant material by alcoholic NaOH varied in exchange capacity from 38 to 178 M.E. (milliequivalents) per 100 grams, while that extracted by aqueous

NaOH varied from 327 to 420 M.E. per 100 grams. It was found that the maximum absorbing capacity of lignin depended upon complete hydrolysis of the complex. This is shown by the fact that the exchange capacities of lignins extracted with alcoholic NaOH were all increased to approximately 200 M.E. when hydrolyzed with HCl, while the capacities of the aqueous soluble lignins were increased to approximately 400 M.E. If we assume that lignin in raw plant material is in part combined with cellulose as lignocellulose this hydrolytic effect of HCl is easily explained.

Throughout the investigations (**3**, **4**), which have been very briefly presented, the fact that lignin is a constituent of most plants was outstanding. This fact suggested that lignins, and possibly other organic compounds, notably the lipoids and pectins, may function in base exchange reactions within the plant. Absorption capacity would be limited by the combination with cellulose. Thus lignin may be said to exist in the plant in both active and inactive forms with the base exchange capacity varying accordingly.

Experimental

To investigate the base exchange phenomenon in plant material, alfalfa, one of the principal crops of the Southwest, was chosen for study. Five-gram samples of air dried, finely ground alfalfa were treated as follows:

1. Without any preliminary washing, duplicate samples were leached with neutral normal barium acetate and neutral normal ammonium acetate, 250-cc. volumes being used. Hydrogen was determined by electrometric titration of the barium acetate leachate and calcium, magnesium, potassium and sodium determined in the ammonium acetate leachate by gravimetric methods.

2. Duplicate samples were leached with 500 cc. of neutral 95 per cent. ethyl alcohol, after which they were leached with barium and ammonium acetates as in 1. The alcohol and acetate leachings were analyzed separately.

3. Duplicate samples were leached with 500 cc. of distilled water and then leached with barium and ammonium acetates as in 1. The water and acetate leachates were analyzed separately.

In each case where ammonium acetate was used, the samples were subsequently leached with alcohol to remove the excess ammonium acetate, and then the absorbed ammonium determined quantitatively. The data are given in table I.

That alfalfa possesses the property of exchanging its bases and hydrogen with other bases in chemically equivalent amounts is shown by a comparison of the total amount of ammonium absorbed and the sum of the displaced bases and hydrogen in column 3. In other experimental work similar data

TABLE I

TOTAL BASES, THE RELATIVE SOLUBILITIES IN ALCOHOL AND WATER, AND THE FIXED EXCHANGEABLE BASES

RESULTS ARE RECORDED IN MILLIEQUIVALENTS PER 100 GRAMS

	TOTAL BASES AND HYDROGEN	INSOLUBLE IN ALCOHOL BUT EXCHANGEABLE	INSOLUBLE IN WATER BUT EXCHANGEABLE
Exchange* capacity	44.1	41.6	39.6
Hydrogen	16.0	Trace	7.0
Calcium	78.0	63.0	27.8
Sodium	49.9	5.7	3.2
Magnesium	11.1	3.7	3.1
Potassium	30.2	3.9	0.15
		Dissolved by alcohol	Dissolved by water
Calcium		11.8	52.5
Sodium		51.1	52.3
Magnesium		9.9	9.7
Potassium		28.4	28.6
Total	185.2	177.5	184.35

* Calculated from the NH_4 absorbed from ammonium acetate.

have been obtained by determining the saturation capacity of alfalfa for barium and calcium ions. Column 1 of table I shows the total amount of bases and hydrogen present both as soluble and as fixed forms. In this case the soluble cations are approximately three times the fixed ions on a milliequivalent basis.

Alcohol was used as a leaching solvent to determine whether or not the soluble unfixed cations could be removed preparatory to determining the fixed cations. It will be noted that the calcium is difficultly soluble in alcohol, while the sodium, potassium and magnesium are largely removed by this solvent. Alcohol, therefore, fails to qualify as a preliminary leaching liquid in the determination of exchangeable bases.

Column 3 closely represents the ratio of bases in fixed and soluble forms, although there is some evidence that a part of the fixed potassium is displaced by soluble calcium during the leaching operation.

In addition to demonstrating an equivalent exchange property of bases, the data show that calcium is the predominant fixed base. The essential need of this high calcium absorption is manifested in the property of the plant material when saturated with calcium and with an univalent base, such as sodium or potassium. Leaching was very rapid with the calcium saturated alfalfa, but when saturated with sodium or potassium the plant tissues became swollen and dispersed, and leaching velocity was greatly reduced. Tissue permeability is therefore a function of calcium saturation and will be reduced if too great a displacement of calcium by sodium or potassium takes place.

Common ion effect

In base exchange reactions the energy of absorption of the exchange complex is of the order Ca > Mg > K > Na and is therefore, for the bases which have been discussed, greatest for calcium. On the other hand, absorption is also influenced by the concentration of the salt of a common base and other electrolytes in the control solution. The property of ionization of basic lignates and the effect of common ions on this property has been demonstrated (**4**). Similar experiments with alfalfa were also conducted.

Six 2-gram samples of alfalfa were leached with neutral 0.5 normal calcium acetate, after which the excess salt was removed by leaching with water. These calcium saturated samples were then placed in 500-cc. volumes of varying concentrations of KCl solution, shaken occasionally for 4 days and the displacing effect of the K ions ascertained by determining the Ca in solution. The results are given in table II.

TABLE II

REPLACEMENT OF CA FROM CA-SATURATED ALFALFA IN CONTACT WITH VARYING CONCENTRATIONS OF KCL

2 GRAMS ALFALFA 500 CC. OF SOLUTION

EXPERIMENT NO.	KCL PER 500 CC.	FIXED CA IN 2 GM. ALFALFA	CA DISPLACED BY KCL
	gm.	*gm.*	*gm.*
1	None	0.0160	0.0016
2	0.5	"	0.0032
3	1.0	"	0.0036
4	2.5	"	0.0041
5	5.0	"	0.0044
6	10.0	"	0.0045

A similar experiment was conducted in which the potassium-saturated alfalfa was prepared by leaching with a solution of potassium acetate and finally with 95 per cent. alcohol to remove excess of salt. These samples were then placed in contact with 500-cc. volumes of varying concentrations of $CaCl_2$ solution, shaken occasionally for 4 days and displaced potassium ascertained by determining the calcium left in solution. The results are given in table III.

These data clearly illustrate the greater energy of absorption for calcium. With a solution of 10 gm. KCl per 500 cc. in contact with 2 gm. calcium-

TABLE III

DISPLACEMENT OF K FROM K-SATURATED ALFALFA IN CONTACT WITH $CaCl_2$ SOLUTIONS
2 GRAMS ALFALFA PER 500 CC. SOLUTION

EXPERIMENT NO.	$CaCl_2$ PER 500 CC.	CA ABSORBED BY ALFALFA	ABSORBING CAPACITY OF 2 GM. ALFALFA IN GM. CA
	gm.	*gm.*	*gm.*
1	0.1	0.00780	0.0169
2	0.5	0.00997	0.0169
3	1.0	0.01490	0.0169

saturated alfalfa, only one-fourth the calcium was displaced. On the other hand, when the potassium-saturated alfalfa was placed in contact with solutions of $CaCl_2$ the displacement of K was almost complete in the presence of 1 gm. $CaCl_2$ per 500 cc. These data lend confirmation to those in table I and illustrate why the absorption capacity of the plant is largely saturated with calcium. Water permeability and turgidity of the tissues apparently demand a near saturation with calcium.

The reactions described, the data presented and the observations which have been made, may be used to explain a number of experimental observations to be found in the literature where mention is often made of the abnormal changes in permeability of plant cells subjected to single salt cultures. According to OSTERHOUT (**5**) the antagonistic effect of salts is due to the presence in the cell of a compound regulating permeability. HANSTEEN-CRANNER (**2**) suggests that lipoid and pectic materials govern permeability of cell walls. Alkalis act as dispersants and calcium as a coagulant. PRIESTLEY (**6**) found a similar property for pectic acids, namely, the formation of gelatinous soluble salts with sodium and potassium, and of insoluble flocculent salts with calcium. These statements agree with our own observations on the property of the alkali- and alkali earth-saturated alfalfa. GENAUD (**1**) has recently demonstrated a rapid ionic interchange between the cellular membrane of yeast cells and the surrounding fluid. The cationic interchange usually involves equimolecular quantities. He also found that bivalent ions penetrate more easily than univalents. The cell membrane and cell vacuole behave like insoluble organic acids, exchanging their cations for those of the environing medium until an equilibrium, due to mass action, is obtained.

It is also of interest to note that STILES (**8**) greatly emphasizes the failure of osmotic views to afford a complete explanation of the cell reaction to its dissolved substances. He observed (**7**) that when storage tissue is immersed in a solution of a single salt, the ions of the salt are not absorbed in equivalent amounts. The balance of the ionic charges in the external solution is maintained by the diffusion of ions from the tissue. He also mentions that salts may combine chemically with the cell constituents. STILES also quotes similar work by REDFREN (7). In the latter's experiments it was found that on immersing tissues in a solution of $CaCl_2$ the calcium was absorbed in excess of the Cl and that Mg and K ions had diffused out of the tissue, being replaced in the tissue by calcium.

These few references serve to show that there is every reason to suppose that certain tissue constituents play a part in the interchange of substances between plant membranes. It is shown by our experiments that absorbed bases may be displaced by other bases or by hydrogen, and that this interchange may play a part in ionic movement.

Summary

Air dried plant tissues of alfalfa show a definite property of absorbing bases or hydrogen and exchanging them for other bases in chemically equivalent proportions. It is suggested that living tissues may exhibit this same property and that it may be related to the phenomenon of the permeability of plant tissues and cationic concentration within the plant system. The diffusion of bases through the plant tissues may be aided by an actual combination of the base with a relatively insoluble anion, such as lignin for example, to form a definite chemical combination, or to form an absorption complex with other electronegative colloids which are tissue constituents.

ARIZONA AGRICULTURAL EXPERIMENT STATION,
TUCSON, ARIZONA.

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RAPIDITY OF RESPONSE OF SPINACH TO CHANGE IN PHOTOPERIOD

JAMES E. KNOTT

When plants are shifted abruptly from existing photoperiodic conditions to a day-length favorable for flowering, changes in the metabolism of the plant result ultimately in flower-bud formation. The change thus initiated should be easier to demonstrate than the gradual shift from a vegetative to a flowering stage which accompanies the natural variation in length of day. These experiments were conducted to see how quickly a change could be noted in the plant after a change in photoperiod.

GARNER, BACON and ALLARD (**1**) found a sharp decline in the hydrogen-ion concentration of the sap of the region of the growing points of a number of species of plants. This occurred three to five days after the plants were transferred to a short day. They believed this to mark definitely the actual transition from the vegetative to the flowering stage.

The catalase activity of the tissues of the growing point has been shown (**3, 4**) to be closely associated with the vegetative or reproductive condition of the bud. As the seed-stalk increased in height the catalase activity decreased. Unpublished data obtained in connection with earlier studies (**4**) indicated that there was a considerable increase in catalase activity of cosmos buds the morning following the sudden change from a long to a short day, although some days later the catalase activity decreased. Further, it seemed that this increase in catalase activity could be noted within a few hours of the change.

A vegetative spinach plant may have a stem height of 5 to 7 mm. as measured from the junction of the root and stem. Thus in data previously presented (**3**) which showed a correlation between the increase in height of seed-stalk and the decrease in catalase activity of the apical bud, there were 43 of the 143 individuals which had a seed-stalk height of less than 10 mm. This stage of development was accompanied by a high catalase activity of the bud. It was impossible to say whether or not this level of catalase activity was higher than it was before the plants were exposed to the longer photoperiod. Seed-stalk development had not proceeded far enough for elongation to be visible. Nor had the bud reached the stage which would be accompanied by a decline in the catalase activity.

Methods

Stock seed of two varieties of spinach developed by the Virginia Truck Experiment Station was planted on September 24, 1930, and January 30, 1931, in a ground bed of the greenhouse. Six 16-foot rows of each variety, spaced 12 inches apart, were planted. Virginia Savoy is a variety which will produce a seed-stalk quickly under favorable conditions. Old Dominion is much slower in changing from the vegetative to the reproductive stage.

The temperature of the house was kept within the range of 55 to 60° F. For a week preceding the start of experiment 1, the spinach had been covered with black sateen at 4:30 P. M. Beginning on the afternoon of December 15th, a section across the eight rows was given a 5.5-hour increase in photoperiod. Four 100 watt bulbs were suspended 30 inches above the plants. The remainder of the bed was covered with a double thickness of black sateen while the lights were in operation. This cloth was moved along and the lights advanced as successive sections of the bed were exposed to the long photoperiod.

In the case of the second planting the whole bed was covered with black sateen each afternoon at such time as to keep the plants under short-day conditions. This cloth was removed about 9 o'clock at night.

To produce the change in photoperiod a strip across the bed was left exposed to the normal daylight, reinforced with artificial light as with the first planting. A screen was provided to prevent the direct sunlight from striking the cloth laid on the plants. Thus heating of the air below the cloth was avoided. The temperature of the house was higher than 60° F. on some of the warm sunny days during April.

Plants were taken from the bed at the times indicated in table I, placed in baskets covered with a damp cloth and removed to the laboratory. The leaves were stripped off until just the small center ones remained. This bud was cut where it joined the stem. One-third as much tissue was used in this experiment as in that with spinach previously reported (**3**). Thus the quantity of fresh tissue varied from 20 to 25 mg. It was macerated with an equal weight of precipitated chalk and 0.1 cc. of distilled water. Sufficient water was added to equal 50 times the weight of the bud tissues. Seven-tenths of a cubic centimeter of the catalase preparation was placed directly in one arm of the reaction tube. Cotton was used to stopper the tubes which were kept in an ice box at 38° F. until 9 o'clock the following morning. The cotton was removed and the contents of the tubes exposed to the air for ten minutes before the catalase activity was determined. One cubic centimeter of neutralized hydrogen peroxide (Dioxogen) was placed in the other arm of the tube. The catalase activity was determined for twelve samples at a time and recorded mechanically by the use of HEINICKE'S apparatus (**2**). The temperature of the bath was kept at 20° C., and the time of agitation was 180 seconds.

TABLE I

RAPIDITY OF RESPONSE OF SPINACH TO CHANGES IN LENGTH OF DAY AS INDICATED BY THE CATALASE ACTIVITY

EXPERIMENT NUMBER	SHIFTED TO LONG DAY BEGINNING	TIME OF SAMPLING	AVERAGE NUMBER OF SECONDS FOR EVOLUTION OF 1 CC. OF OXYGEN		INCREASE IN CATALASE ACTIVITY IN LONG-DAY PLANTS
			SHORT-DAY PLANT	LONG-DAY PLANT	
1	Dec. 15 4:30 P. M.	Dec. 17 9:00 A. M.	A 20.0 ± 0.45*	10.7 ± 0.13*	9.3 ± 0.48
			B 47.1 ± 0.96	35.4 ± 1.22	11.7 ± 1.55
2	Dec. 16 4:30 P. M.	Dec. 18 9:00 A. M.	A 29.6 ± 0.85	25.2 ± 0.71	4.4 ± 1.11
			B 29.4 ± 0.76	26.4 ± 0.39	3.0 ± 0.85
3	Dec. 18 4:30 P. M.	Dec. 19 9:00 A. M.	A 29.1 ± 0.99*	14.7 ± 0.15*	14.4 ± 1.00
			B 28.3 ± 0.63	25.0 ± 0.44	3.3 ± 0.77
4	Dec. 21 4:30 P. M.	Dec. 22 9:00 A. M.	A 41.5 ± 1.06	23.4 ± 0.24	18.1 ± 1.09
			B 52.1 ± 1.53	22.9 ± 0.53	29.2 ± 1.62
5	Dec. 23 4:30 P. M.	Dec. 23 7:00 P. M.	A 25.3 ± 0.68	22.4 ± 0.72	2.9 ± 0.98
			B 27.1 ± 0.64	36.1 ± 1.08	-9.0 ± 1.26
6	April 28 3:00 P. M.	April 28 5:30 P. M.	A 112.7 ± 3.45	67.7 ± 2.68	44.9 ± 4.37
			B 139.5 ± 2.29	118.2 ± 3.91	21.3 ± 4.54
7	April 30 3:00 P. M.	April 30 5:30 P. M.	A 64.8 ± 1.92	53.7 ± 0.90	11.1 ± 2.12
			B 59.4 ± 1.73	54.2 ± 1.64	5.2 ± 2.38
8	May 1 3:00 P. M.	May 1 5:30 P. M.	B 57.1 ± 1.18	57.7 ± 0.83	-0.6 ± 1.44
9	May 4 3:00 P. M.	May 4 5:30 P. M.	B 66.4 ± 1.59	58.9 ± 1.23	7.5 ± 2.01

* Seconds per half cubic centimeter instead of one cubic centimeter.

A = Virginia Savoy, B = Old Dominion.

Discussion of results

The results of these experiments are summarized in table I. Not all of the plants were equal in size, although selected as nearly as possible for this characteristic. Therefore there is some variation in their catalase activity.

The catalase activity was measured for 10 to 18 plants from the short- and the long-day lots of each variety at each time of sampling. Of the 357 determinations there were only 19 in which as many as 3 of the 12 cubic centimeters of oxygen available in the Dioxogen were released. There were 88 which gave as much as 2 cubic centimeters. In most cases the time for the evolution of 1 cubic centimeter of oxygen appeared to be the best basis of comparison, but in the two cases indicated in the table, it was necessary to use the time for one-half cubic centimeter.

The data in table I indicate that there is an increase in the catalase activity of the apical bud of spinach following a change from short to long photoperiod. This increase is evident 16.5 and 40.5 hours after the change. The change is more marked in Viriginia Savoy than in Old Dominion, except in experiment 4.

It will be noted that the catalase activity of the short-day plants has fluctuated from one experiment to the next, due probably to the application of water to the bed, as this was supplied as the soil dried. Thus, more was needed on sunny days, and the daily application of water could not be constant. The two days preceding the start of experiment 6 were cloudy and water was applied the morning of April 28. This appears to be the reason for the difference in the level of catalase activity as compared to that in experiments 7, 8 and 9 where water was applied 30 hours before the start of each experiment. The plants had almost full sunlight the day before and the day of each of these last three experiments.

Experiments 5 to 9 were designed to check the earlier observation with cosmos which suggested that the sudden change in day length might influence the catalase activity within a few hours.

In experiment 5 the increase in catalase activity in Virginia Savoy is not quite great enough to give odds of significance, but indicates that there had been a disturbance of the metabolism. The decrease in Old Dominion in experiment 5 can be explained by the condensation of water on the bud leaves as these plants stood in a moist atmosphere at a temperature slightly above freezing before they were macerated. Although an attempt was made to dry them by touching the surface with dry cheesecloth, enough water remained to cause error in weighing and consequently in the degree of dilution.

Experiments 6 to 9 show that the Virginia Savoy variety has shown a greater increase in activity 2.5 hours after the sudden change in photoperiod, than has Old Dominion. Only in experiments 6 and 9 is the increase in catalase activity of Old Dominion significant.

Plants which had been exposed to the long photoperiod from December 15 and December 24 were measured for seed-stalk height on January 5, 1931. These data are presented in table II and show that it took 21 days for the Old Dominion to reach the height of seed-stalk attained by Virginia Savoy in 12 days under the temperature conditions prevailing. At the end of 12 days, Old Dominion had scarcely started to elongate, as the base of the plant itself may be 5 to 7 mm. high. These figures indicate why a more pronounced and more rapid change in catalase activity might be expected in Virginia Savoy.

TABLE II

AVERAGE HEIGHT OF SEED-STALK OF VIRGINIA SAVOY AND OLD DOMINION SPINACH AFTER CERTAIN PERIODS OF EXPOSURE TO A LONG PHOTOPERIOD

VARIETY	NUMBER OF DAYS OF EXPOSURE TO LONG PHOTOPERIOD	
	12 DAYS HEIGHT OF SEED-STALK	21 DAYS HEIGHT OF SEED-STALK
Virginia Savoy .	38.75 ± 2.397 mm.	90.34 ± 6.411 mm.
Old Dominion .	10.00 ± 0.675 mm.	39.58 ± 1.569 mm.

The increase in catalase activity of the apical bud soon after a change to a photoperiod favorable for flowering is followed later by a decrease. The hydrogen-ion concentration of the apical tissue has been shown to decrease and then increase under similar conditions (**1**). However, the change in catalase seems to occur more quickly. The two may be independent, but simply manifestations of the change in metabolism occurring in the shift from a vegetative to a reproductive type of growth.

Summary

Determinations of the catalase activity of 20 to 25 mg. of fresh apical bud tissue of spinach plants, taken 2.5, 16.5 and 40.5 hours after a change to a longer photoperiod, show that the plants have responded to the stimulus by an increase in the catalase activity. This indicates that spinach has responded rapidly to the change in day length.

The Virginia Savoy variety which produces seed-stalks more quickly than the Old Dominion variety under a favorable photoperiod has shown a greater change in the catalase activity.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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MODE OF OCCURRENCE OF CAOUTCHOUC IN THE GUAYULE, *PARTHENIUM ARGENTATUM* GRAY, AND ITS FUNCTION

FRANCIS E. LLOYD

(WITH ONE PLATE)

The account which I published in 1911 (9) of the mode of occurrence of caoutchouc in guayule, *Parthenium argentatum* Gray, is incorrect. I was led into error by inadequacy of both method and point of view. The purpose of the present paper is to set the matter right, so far as I now understand it.

In the guayule, as in some other rubber-bearing plants, the rubber occurs in the parenchyma cells and is thus segregated. In contrast with this condition is that in the so-called latex-bearing rubber plants, such as *Hevea*, *Manihot*, *Ficus*, *Euphorbia*, *Scorzonera*, *Chondrilla*, etc., in which the rubber is a constituent, more or less prominent according to the species, of a white or colored milky fluid, which is stored in tubes from which, when opened, the fluid flows more or less freely. The fluid in question is always a complex emulsion of rubber[1] and other substances (resins, fats) as the internal phase, with water containing proteins, salts, sugars, etc., in solution as the external phase. The size of the suspensoids differs greatly, and is from 2 microns and less in *Hevea* and *Kickxia* to 60 microns or more in *Musa*. This general statement may now be extended to the guayule for, as will be shown, the rubber associated with other substances occurs in the same manner. The fluid here is equally a latex[2] confined to individual cells. In *Hevea*, etc., it occurs originally in individual cells which break down in series to form tubes, or, as in the seed, in cells which tend to form inarticulate tubes (Parkin, **15**), such as those which occur in many other plants, *e. g.*, *Argemone* (Trecul, **18**).

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[1] Latex is, of course, not always rubber-bearing (see MOLISCH, 14; LLOYD, 12).

[2] The word "latex," while meaning merely a "fluid," has come to be used more specifically for milky rubber-bearing fluids occurring in tubular structures (Oxford Dictionary). It is, however, impossible to adhere to this usage rigorously. For example, the milky exudate from cut surfaces of *Musa* is always spoken of as latex in the more restricted sense; as a matter of fact, this latex is merely the sap (latex in the original sense) of large barrel-shaped cells placed in series like a string of beads, each cell separated from its neighbor by a thin circular wall (double, of course). On cutting fresh living tissue, the internal pressures break down the walls, hence the exudate. If the part cut is fully wilted or has otherwise lost its turgor (as when a banana fruit has been "chilled" (LLOYD, 11) no exudation takes place. The word "latex" is here obviously misapplied unless used in its primary sense. I should prefer, therefore, to adhere to the primary meaning, coupled with qualifying terms, as parenchyma latex (*Parthenium*, *Musa*), vascular latex (*Euphorbia*, *Hevea*). It is well understood the latex vessels of *Hevea* arise by the resorption of the contiguous walls of seriate members. "Sap" could be restricted to the fluid of wood vessels.

The evidence as to the nature of the parenchyma latex of guayule is very simple. If a living plant stem be cut across and the cut surface be dipped into a drop of water on a glass slide and examined at once, the water will be observed to have become milky. Placed under the microscope, it is seen to be a rich suspension of minute particles in lively Brownian movement. If now a section be cut, thick enough to afford uncut cells but thin enough so that uncut cells do not overlie each other, and examined microscopically, the contents of the cells appear as a densely milky fluid, more especially if observed in reflected light with the dark field condenser. In transmitted light, the contents of the cells appear cloudy and more or less obscure, according to their density. At the same time, one may observe that the minute particles or suspensoids in the fluid in each cell are in Brownian movement, the observation of which is most easily made in cells which are not too fully packed with the suspensoids, for obvious reasons. A series of preparations made from a shrub containing poorly to richly loaded cells will afford a variety of pictures, from cells containing only a few minute suspensoids to those containing a very dense suspension. Such preparations, if photographed with a time exposure, give a curious result pictorially, which of course could be expected, namely, cells appearing as if filled with an ill-defined cloud (plate II, figs. 1, 3). It is only when a rapid exposure is used that the individual particles may be resolved, for which purpose not too well filled cells are best (plate II, fig. 5). An instantaneous photograph of diluted cell fluid affords a picture in which one may obtain an impression of the range of size of the particles, which extends from two microns to those of so minute a size as to be just within the limits of resolvability (plate II, fig. 8). The particles are all spherical, as in the latex of *Ficus*, and not pear-shaped, as are many of the suspensoids of *Hevea*. It is not, of course, to be supposed that all the particles are caoutchouc in guayule any more than in other rubber-bearing plants. But that a part of them, indeed the greater part, is caoutchouc may be proved by the use of suitable solvents for other possible components, such as resins, proteins, oils. Sections treated with such solvents for sufficient periods of time still reveal suspensoids remaining undissolved, provided only that a solvent for caoutchouc has not been used. If, however, for such tests cells are used which contain a great deal of rubber, this will have become coagulated. If, on the other hand, cells with a small amount are used, the particles may remain discrete though they usually become more or less adherent to various surfaces present—the mere result of space relations.

If now coagulating agents are used at once, the coagulation of the latex within the individual cells proceeds and there results within each cell several coagula if the amount of rubber is small—that is to say, flocculation takes place (plate II, figs. 4, 6); or, if there is enough rubber, a single clot is formed (plate II, figs. 2, 7). This, as is well shown in the figures in my monograph (1911), is not homogeneous internally, but contains inclusions of fluid—are vacuolated, so to speak—and particles of non-rubber constituents (plate II, fig. 7). The change is irreversible.

The above described behavior in the guayule is of interest both theoretically and practically. From the point of view of physiology it was difficult to regard the caoutchouc, if laid down in the parenchyma cells as solid masses, as a food or other reserve. The question of the function of latex, or, to particularize, of any or all of the constituents of the latex, has puzzled every one who has taken any theoretical interest in rubber plants, for the reason, if for no other, that if any or all of these constituents are physiologically useful, there might be expected some variation in the composition of the latex at one time or another in the cycle of growth, let us say, and thus during the year the crop of latex would be uneven in quality or quantity or both. While in the case of *Hevea* there seem to be minor differences in composition of the latex as between tree and tree, or more obviously between race and race, the differences are slight and appear to indicate nothing in regard to physiological significance. There is also the now known effect of enrichment of the soil by sodium nitrate, etc., in increasing the yield of latex (GRANTHAM, **6**), but it can be inferred that this effect is general, affecting many if not all features related to growth. Possibly the number of stone-cells in the cortex would be affected, but this feature is so different quantitatively in various races of *Hevea* that it would require hardihood to claim a physiological function for the stone-cell. Some would perhaps argue for a protective effect, but there is no evidence that this is the case: just as a similar effect has been argued for latex (DE VRIES, **19**) in the sealing of wounds, without any real evidence that *Hevea* is less subject to the ills of the flesh than other non-latex producing plants. However, it may be suggested that the coagulative capacity of rubber and other latices serve for the prompt closure of wounds so that the internal pressures, which are high (MOLISCH, **14**) may be restored and preserved.

As to the experimental evidence in support of the idea that latex is in part at least a fund for energy supply, there is the evidence adduced by FAIVRE (**3**, **4**, **5**) for *Tragopogon porrifolius, Morus alba,* and a *Ficus.* By varying conditions (including absence of carbon dioxide) he observed impoverishment of the latex in the above forms, and inferred their nutritive value. His views were favorably received by DE VRIES (*l. c.*, **19**). CH. BERNARD (**1**) also did experiments with *Euphorbia thymifolia* and *E. splendens* exposed to CO_2-free air and observed the corrosion of the starch grains; and what happens to one component of the latex may happen to another. **Pfeffer** (**16**), however, asserts that among other substances India rubber is "incapable of further metabolism," which remark, whether eventually shown to be true or not, serves to indicate the crux of the problem for us at the moment. The only additional experimental evidence that is available is contained in a note by D. **Spence** (**17**), who states that in a young *Ficus elastica* "grown in an atmosphere and soil free of carbon dioxide, gradually drew on their milk which became nothing more than water after a few weeks' time." This experiment, supporting the conclusion of **Faivre** (**3**), indicates that in the absence of a raw material for the elaboration of a hydrocarbon, here caoutchouc, the supply already present may be metabolized. The presence of suitable enzymes, shown in the first instance by **Spence** (**17**) for *Hevea* and *Funtumia,* coupled with the great surface area of the suspensoids, lends support to the idea. The very condition in which the latex occurs, namely, in a vascular system, or system of tubes, their very wide distribution in the plant kingdom, and the evident nutritive values of many of the latex constituents predispose only to the view that, in general, latex is not merely a depository of wastes.

In considering the guayule and other plants which have and hold their caoutchouc in like manner, namely, in the individual cells of the parenchyma, the predisposition is to compare this condition to analogous ones in which known metabolically useful materials are accumulated, *e. g.*, starch in the potato tuber. In the latter it is easy to demonstrate experimentally that the starch is actually and measurably used. Now that we know that the caoutchouc occurs as a colloidal suspension, precisely as in the latex of *Hevea,* etc., we have no difficulty in accepting the possibility of its attack by enzymes: what seemed difficult, holding the older and incorrect idea that the caoutchouc is disposed in solid masses, a single one in each cell, is now easy when the surface relations inherent in a colloidal suspension are realized (**Molisch**, **14**, p. 80). On general principles there is no particular difficulty in expecting even so refractory a substance as rubber to be acted upon readily if the proper agencies (enzymes) are present. This would be merely the reversal of the anabolic process. This argument has been used by **Molisch** (*l. c.*) in regard to the particulate condition of the other substances in latex.

That, however, the hydrocarbon in question is, in the case of the guayule, actually a reserve substance and is as actually made use of in metabolism requires proof, which has not yet appeared. We know that during periods of rapid growth very little caoutchouc is formed, this happening after the cessation of growth during the drought period following the rain which made growth possible. The amount of rubber which can occur in maximum quantity (*ca.* 20 per cent.) depends not alone on the particular race of guayule (**McCallum**, **13**), but also on the rapidity of growth and the length of the drought following cessation of growth. At all events, in the semi-arid deserts in which it occurs, the guayule accumulates rubber until some maximum is reached and the amount then appears to remain constant until a new growth period sets in. The addition of new tissues during the growth period makes it very difficult to determine whether meanwhile the caoutchouc is being drawn upon as a source of energy. The solution of the problem is one of the most interesting in the field of plant metabolism and it is to be hoped that those favorably placed and interested will find it possible to find the solution.

The view that the caoutchouc in the guayule confers on the plant some ability to withstand drought has been advanced from time to time, but no convincing evidence has accrued. That the plant wilts at once on being pulled suggests a negative answer. On the other hand, plants so situated that they must withstand a drought period are subjected to a very gradually effective drying out which might affect the leaves without affecting the stems, while these may have a superior drought resistance conferred on them by the presence of the caoutchouc. The fact that it requires a considerable period of time after the shrub has been "pulled" before it "cures" so that it can be milled might be taken as an indication that during some period—probably a short one—the parenchyma cells retain their vitality as indicated by the dispersed condition of the contents of the cells.

On the other hand, closely related species, such as the mariola (*Parthenium lyratum*), which appear to be equally able to withstand drought, if not more so, have very little caoutchouc; but here we impinge on one of the very difficult problems in the field of adaptation, the question of how far the same ends are attained by different means.

An additional suggestion is prompted by the recent work of WALTER (**20**), who reports that drought resistance of leaves (the stems were not investigated) "varies directly with the maximum osmotic value and with the osmotic inertia of the plant," namely, that the accumulation of caoutchouc and water-soluble substances such as oils and some resins may increase the osmotic value of the sap merely by occupying space, thus by indirect means gradually changing this from a lower value during the continuation of drought.

The practical interest attaching to the matter of the condition of the caoutchouc is well understood by those who during the past twenty-eight years have been concerned in the extraction of rubber from the guayule shrub. It has long been known in northern Mexico that by merely chewing the stems, rubber ("hule") could be obtained, and when communal methods were used a sufficient amount could be obtained to make balls for playing games. Rubber was well known to the prehistoric peoples of Mexico, who made small images, utensils and balls, etc. It was obtained by coagulating the latex of *Castilloa;* and it is not unlikely that such objects found their way as far north as southern Arizona (LLOYD, **10**). It is, however, pretty certain that in northern Mexico the rubber of guayule was also used (LLOYD, **9**). At any rate, the method of obtaining small quantities by mastication is and has been general knowledge among the peons of northern Mexico for a long time, certainly previous to the beginnings of the manufacture of guayule rubber just previous to 1903. It was in this year that Mr. WILLIAM A. LAWRENCE discovered that this substance could be extracted from the shrub by a strictly mechanical process of comminution and flotation in water, by which the floating rubber could be separated from the bagasse (CARNAHAN, **2**). For many years thereafter, however, there were measurable and even serious losses due to various imperfections in the methods of manufacture, following from imperfect knowledge of what constituted "curing" the shrub, which we now know to be the time and conditions for bringing all the suspensoids into the coagulated condition.[8] With the fact before us that the rubber in the guayule exists as a colloidal suspension, experimentation can proceed with a perfectly definite criterion before the investigator. It is one so simple, moreover, that it is a matter of surprise that it was not long ago discovered, namely, the method indicated above. One needs but to make a cut across the tissues and touch the cut surface to a drop of clean water on the piece of glass. If the rubber exists as suspensoids, they will flow out and render the water milky. Slight turbidity may be determined by putting a few slices of tissue in a small volume of water and then determining the turbidity by means of a strong beam of light (TYNDALL effect). For the purpose of checking this method of treatment, the dried material, after allowing a drop to dry on the glass, may be treated with acetone to remove the resins, and the adhering caoutchouc then examined, when, of course, staining may be used.

At this point I wish to acknowledge the courtesies extended by Mr. GEORGE H. CARNAHAN, President of the Inter-Continental Rubber Company, who made it possible to re-examine living guayule, both at Salinas, California, and at Torreon, Mexico, in 1926 and 1927. It is with his concurrence that the above notes are published.

MCGILL UNIVERSITY,
MONTREAL, CANADA.

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[8] Since such plants as *Scorzonera* and *Chondrilla*, certain species of which are being exploited by the Russians, are vascular latex plants, and are hardly susceptible of being tapped, the latex in them must be allowed or caused to coagulate, before extraction, thus following the method of "pilonage" used in Africa for the extraction of the rubber from *Landolphia heudelotii* (JUMELLE, 7); that is, by a method fundamentally similar to that used for guayule.

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EXPLANATION OF PLATE II

FIG. 1. Section of the living cortex, including a portion of a resin canal, of *Parthenium argentatum* Gray. The cells appear as if filled each with a dense cloud. This is the result of a time exposure on many suspensoids in Brownian movements. Compare with figure 5.

FIG. 2. The same section after brief treatment with weak acetic acid. The latex within the cells has now been coagulated in nearly solid masses, almost filling them.

FIG. 3. Living cells from the pith, time exposure. The cloudy appearance of the interior of the cells is owing to the movement of the suspensoids, as in figure 1.

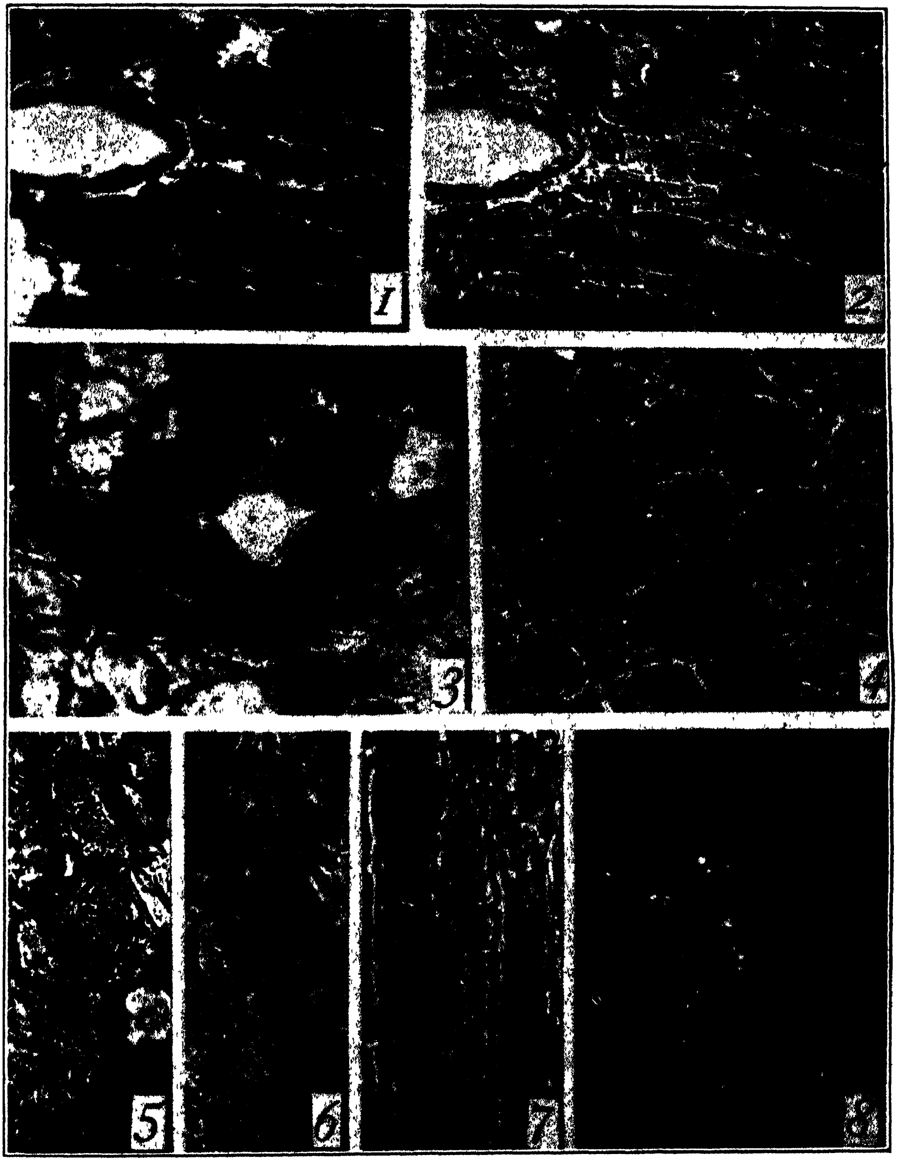
FIG. 4. The same section as in figure 3, after treatment with acetic acid. The amount of material in the form of suspensoids is not great enough to make a solid mass, but is merely flocculated. Contraction follows later.

FIG. 5. Instantaneous photograph of three undamaged cells of the cortex, showing the suspensoids of caoutchouc (for the most part). A time exposure would have yielded a result such as in figures 1 or 3.

FIG. 6. The same after treatment with acetic acid.

FIG. 7. A larger picture of such cells as are seen in figure 2 showing that after coagulation the coagula are not homogeneous.

FIG. 8. Dark-field photograph of the parenchyma latex diluted with water.



LLOYD: CAOUTCHOUC IN GUAYULE

OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION DURING THE GROWTH OF YEAST

OSCAR W. RICHARDS AND FLORENCE W. HAYNES[1]

(WITH THREE FIGURES)

In the present experiments the oxygen consumption and carbon dioxide production of a pure strain of yeast have been investigated throughout the first and second cycles of its growth in a synthetic nutrient solution. Few measurements of the respiration of yeast in the literature are comparable to these observations, since other investigators have used different kinds of yeast under various experimental conditions which have not always been stated clearly. The measurements have usually been made only for short intervals of time. COPPING (**2**) has published curves for the oxygen consumption, carbon dioxide production and cell count of *Saccharomyces cerevisiae* on different media for a period of about four days but readings were made only at daily intervals and no attempt was made to correlate the gaseous exchange of the yeast with its growth.

For these experiments a pure strain of *Saccharomyces cerevisiae* Hansen previously obtained from a single cell (RICHARDS, **5**)[2] was grown in WILLIAM'S medium.[3] The seedings were made with 0.10 cc. of a suspension from yeast cultures of about the same age and the technique of measuring the multiplication of the yeast was that of RICHARDS (**6**).

The oxygen consumption and carbon dioxide production of the yeast were determined with differential microrespirometers of the type devised by THUNBERG (**9**) and more recently described by FENN (**3**). For the measurements of oxygen consumption 0.5 cc. portions of 20 per cent. sodium hydroxide were introduced into small cups in the two chambers of the respirometer to absorb carbon dioxide. Into one chamber was placed 1 cc. of medium containing yeast and into the other enough water to make the air spaces equal. As oxygen was consumed the index drop in a horizontal capillary connecting the two chambers was drawn toward the chamber containing the yeast. The oxygen consumption was obtained by multiplying the movement of the drop in centimeters per minute by the volume of the capillary in cubic centimeters per centimeter. Twice this value was used since the second, compensating chamber reduces the movement of the drop by one-half. When measuring the carbon dioxide production the sodium hydroxide was omitted, so that the value for the CO_2/O_2 was obtained directly. The addition of the amount of oxygen (O_2) consumed to this value gives approximately the carbon dioxide (CO_2) produced.

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[1] The measurements of respiration were made by Miss HAYNES and the growth counts by RICHARDS.

[2] Isolated from a cell of yeast no. 2335 furnished to RICHARDS through the courtesy of Dr. F. W. TANNER.

[3] Sucrose 20 gm.; $(NH_4)_2SO_4$, 3 gm.; KH_2PO_4, 2 gm.; asparagine, 1.5 gm.; $CaCl_2$, 0.25 gm.; $MgSO_4$, 0.25 gm.; distilled H_2O, 1,000 cc. All chemicals were of Merck's Blue Label reagent quality.

The apparatus was suspended in a water-bath in which the temperature was maintained between 28.2° and 28.3° C. About every 12 hours cell counts were made on control tubes in the same bath. Carbon dioxide was not removed from these tubes with sodium hydroxide because previous experiments[4] had shown that such a removal of carbon dioxide did not significantly influence the growth of the yeast. The cotton stoppers were not tight enough to prevent the escape of the gas. The growth curve is an average curve. As the yields in the several respirometers varied slightly owing to the technical difficulty of getting exactly the same seeding in each, the readings were brought together at the end of the growth period (*cf.* RICHARDS, **6**).

The experiments lasted approximately 130 hours and readings were taken every 2 to 5 or more hours. The oxygen consumption was averaged over the intervening period. Because of the large amounts of carbon dioxide produced during the fermentative phase of the yeast growth, measurements of the rate of carbon dioxide produced were made every few hours for short

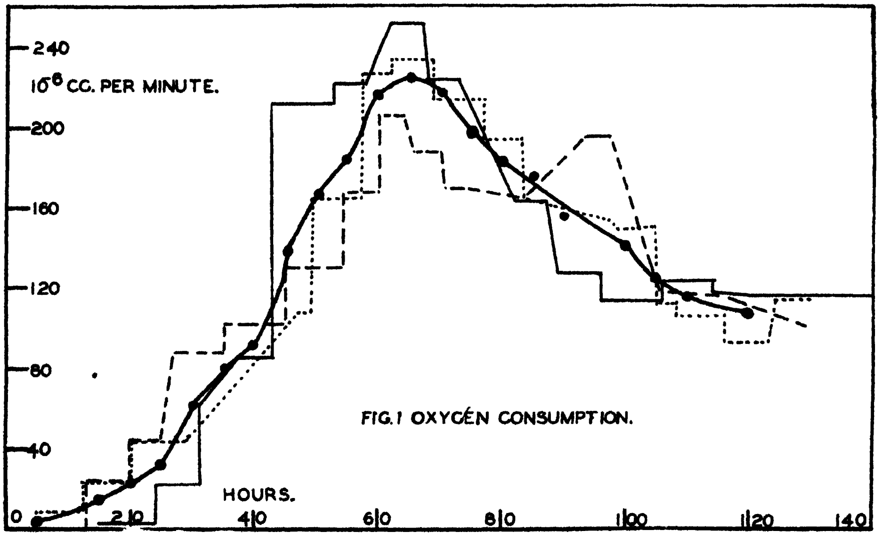

FIG. 1. The oxygen consumption of the cultures of yeast.

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[4] The slight increase in the growth of the yeast when the CO_2 was removed by suspending a short vial containing NaOH above the culture medium was less than the variation of the crop in the respirometers.

intervals of time. As the chambers were frequently opened to the air in order to return the index drop to its initial position, there was probably no oxygen depletion. After these manipulations time was allowed for the equilibrium to become established before making measurements.

The oxygen consumption is plotted in fig. 1 from a series of three experiments. The determinations of the individual experiments (averaged over 3–13-hour periods) are plotted as horizontal lines. These are joined by vertical lines to show the parts of a single experiment. The continuous curved line is the average curve obtained by drawing the curve through average points taken at 5-hour intervals. The rate of oxygen consumption, shown by this average curve, increases until about 65 hours after seeding and then decreases until it reaches an equilibrium at about 130 hours.

The rate of growth, fig. 2, is greatest at about 30 hours, returns to zero at the equilibrium between the first and second cycles of growth (65–70) hours), and then increases to a lesser extent during the second cycle. From a comparison of the two figures there appears to be no direct correlation between the rate of growth and the rate of oxygen consumption which would suggest that the oxygen consumed is not used primarily to supply energy directly to the cells but for the oxidation of products in the medium. The oxidation of the products in the medium and the release of materials

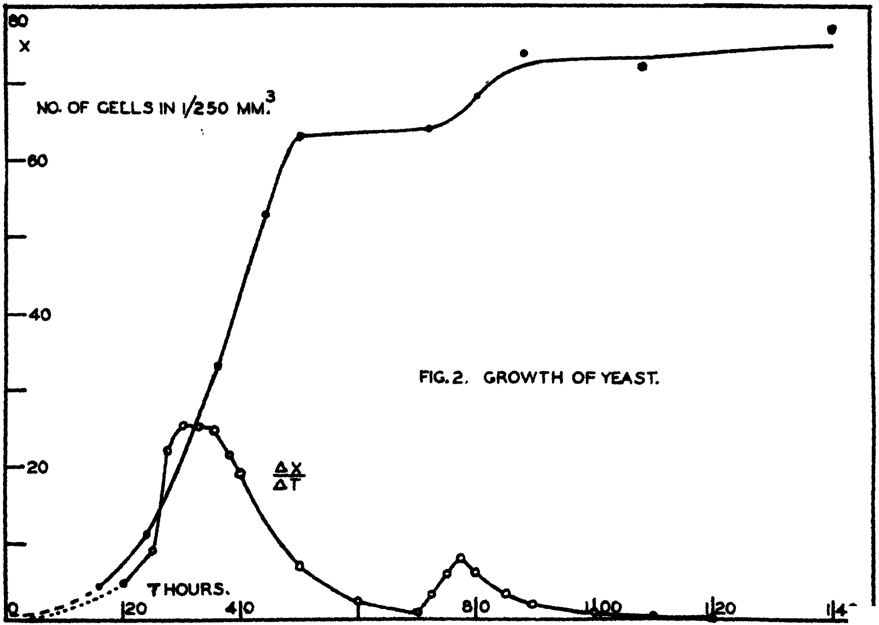
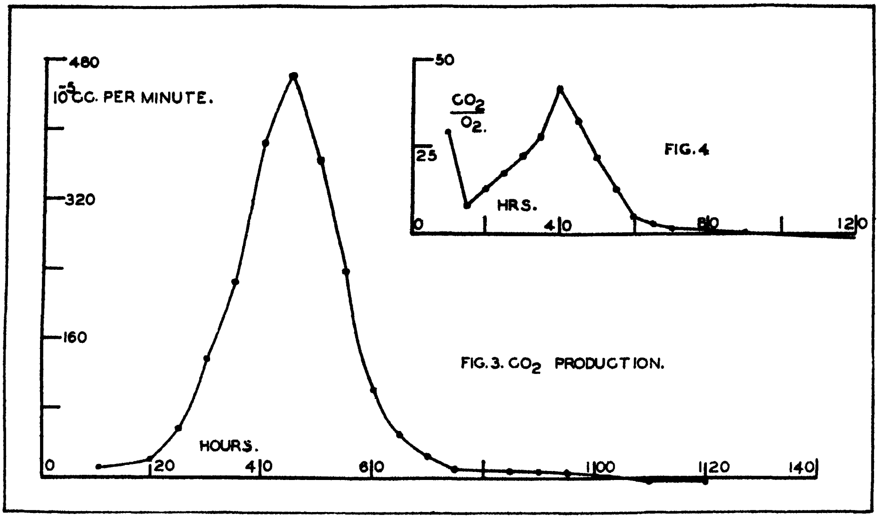


FIG. 2. The average growth curve of the yeast and the rate of the growth.

into the medium from the disintegration of the dead cells seem to increase the fitness of the medium for the yeast. The population then increases (second cycle of growth) because less cells are killed. The percentage of cells budding shows little regular change during the two cycles of growth.[5] The oxygen uptake for the first few hours occurs gradually, indicating that the "bottom" yeast used in this investigation did not absorb the oxygen as did the "top" yeast used by Brown (**1**).

A curve showing the rate of carbon dioxide production of a series of four experiments appears in figure 3. To obtain this curve ordinates from an average curve for the observed values of CO_2-O_2 were added to the ordinates of the corresponding O_2 consumption curve. Since the values of individual experiments are in good agreement only the average curve is plotted. The facts that the scales in figures 1 and 3 are different, and that the carbon dioxide produced greatly exceeds the oxygen consumed, must be noted when comparing those two figures.



Figs. 3 and 4. The average carbon dioxide production of the yeast. Fig. 4 (insert). The respiration quotient of the yeast.

The rate of carbon dioxide production increases rapidly until about 45 hours after seeding and then decreases until an equilibrium is reached at 70 hours. The decrease in carbon dioxide production precedes the equilibrium in growth at the end of the first cycle. The carbon dioxide probably omes largely, if not entirely, from the fermentation of sugar.[6] The yeast used in these experiments is a bottom yeast and is growing in a medium favoring growth rather than fermentation. The products of fermentation decrease the population by selectivity, eliminating the larger buds before they can become resistant (RICHARDS, 8). Consequently, the resulting waste products negate somewhat the energy derived from the fermentation.

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[5] A detailed description of the changes in the culture medium will be published by Richards.

[6] About 75 per cent. of the sugar in the medium is used up by 60 hours. (*Cf.* also Richards, 7.)

In view of the great amount of work on the respiratory quotient of cells and tissues it is of interest to calculate the values of CO_2/O_2 for yeast. The curve, fig. 4, plotted from these values reaches a maximum of 41.7 at 40 hours and then decreases to zero.[7] Toward the end of the second cycle glycogen is stored in the cell and the sugar in the medium is almost gone. There is, however, some change in the nitrogen content of the medium indicating metabolism of this material. This prevents any simple measure of the energy used by the cell from the oxygen entering the medium and the carbon dioxide liberated from the medium.

A series of experiments at a lower temperature (about 20° C.) indicates that the highest points on the oxygen and carbon dioxide rate-curves appear later and are reached more gradually than at 28°. The changes associated with different temperatures have not yet been sufficiently studied to warrant a more detailed comparison at this time.

Summary

1. The curves for the rate of oxygen consumption and carbon dioxide production of a population of yeast have been compared with the first two cycles of growth during 130 hours after seeding the culture. The cells were grown in WILLIAM's medium at 28.2–28.3° C.

2. The growth rate in the first cycle in these experiments is greatest at 32 hours and the population reaches an equilibrium number at 60 hours. At about 72 hours the population of cells increases again until a second equilibrium is reached at about 90 hours. The rate of growth during the second cycle is greatest at 78 hours.

3. The rate of oxygen consumption reaches its maximum at about 65 hours after seeding during the equilibrium between the first and second cycles of growth. This suggests that the oxygen is used mainly to oxidize products in the culture medium. The rate of oxygen consumption decreases slowly and tends to reach an equilibrium at the end of the experimental period. The production of carbon dioxide is very great during the first cycle of growth (25–60 hours after seeding) but decreases as the sugar of the medium is fermented.

OSBORN ZOOLOGICAL LABORATORY,
YALE UNIVERSITY, NEW HAVES, CONNECTICUT, AND
THE MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASSACHUSETTS.

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[7] This is a little less than the values of 60–100 given by MEYERHOF (4), and would place this yeast intermediate between the beer and the pressed yeasts.

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EFFECT OF HOT-WATER TREATMENT ON THE CARBOHYDRATE CHANGES IN NARCISSUS BULBS DURING STORAGE

HEINRICH HASSELBRING

Introduction

Since the work of RAMSBOTTOM (**17, 18**) in England and VAN SLOGTEREN (**20, 21, 22**) in Holland, who adapted and applied the Jensen hot-water treatment for grain smuts to narcissus and other flower bulbs for the destruction of eelworms, this treatment has come into wide-spread use for the destruction of animal parasites in dormant bulbs. In accordance with the disinfection requirements which have been in effect in the United States since January 1, 1926, all narcissus bulbs imported into this country and also those entering into interstate commerce are subjected to this treatment unless, after requisite inspection, they have been found free from bulb-fly larvae and eelworms.

Aside from the destruction of eelworms and other animal parasites, European experience seems to indicate that the hot-water treatment itself has a stimulating effect on the growth of the bulbs (**23**), so that, according to VAN SLOGTEREN (**24**), this treatment, first reluctantly employed in the combating of parasites, is now annually applied in Holland to large quantities of perfectly sound bulbs. In the United States also there is a current impression among florists that hot-water treatment, which does not result in direct injury to the bulbs, brings about an increased vigor. GRIFFITHS (**6**), however, who also has noted this effect, is inclined to the belief that the favorable result is attributable entirely to the destruction of bulb mites.

Some time before the hot-water treatment of bulbs for the destruction of animal parasites came into use it had become a common horticultural practice to immerse dormant plants in warm water for short periods in order to force them into early growth and flowering (**10**). This treatment has been found by MÜLLER-THURGAU and SCHNEIDER-ORELLI (**13**) to be followed by changes in the relative quantities of the different carbohydrates within the treated tissues.

While the essence of the hot-water treatment is the same, whether applied to plants for the purpose of breaking the rest period or to bulbs for the destruction of parasites, there is a difference in the subsequent handling of the material. Plants or plant organs treated for the purpose of breaking the rest period are placed under forcing conditions immediately after the treatment, whereas bulbs which at some time during their summer dormancy are treated for parasites usually remain in storage for a time before they are planted. It was of interest, therefore, to study the carbohydrate transformations in treated and in untreated bulbs during storage in order to determine whether the hot-water treatment of bulbs which subsequently remain dormant for a time produces on the carbohydrate constituents any persistent effect which might be correlated with the later behavior of the plants during growth.

The work was carried out with the cooperation of Dr. DAVID GRIFFITHS, Senior Horticulturist in charge of Bulb-Culture Investigations, in the Bureau of Plant Industry, U. S. Department of Agriculture. Through Doctor GRIFFITHS it was possible to obtain narcissus bulbs of constant variety and known cultural history, which were practically free from parasites and diseases.

Preparation and mode of storage of the bulbs

The bulbs used in this work were of the self-colored trumpet variety King Alfred of *Narcissus pseudo-narcissus* L. They were harvested at the Arlington Experiment Farm, at Rosslyn, Virginia, on June 28, and allowed to dry in a loft until July 8. On that day they were freed from tops, roots, and loose scales. The following day they were washed and spread out on large-meshed wire screens to dry over night in the breeze from electric fans. The next morning, July 10, the bulbs were sorted into lots of 45 each, in such manner that each lot graded as nearly alike as possible, from the largest to the smallest bulb. The lots, weighing about 2.2 kilograms each, were put into mosquito-net bags, which were spread out in a single layer on wire screens in the storage room. The next day, July 11, after the material had come into equilibrium with the room temperature, the bags were weighed. On July 12 the first set of four bags was taken to the laboratory and prepared for analysis. Every two weeks thereafter four of the remaining bags were used for analysis. All the bags in storage were weighed every two weeks in order that the carbohydrate changes might be based on the original weight of the bulbs.

On August 10, the day after the third sampling, one-half of the remaining bags was heat-treated by submergence for three hours in a stirred water-bath at a constant temperature of 43.5° C. These bags were then hung up over night to dry in the breeze from electric fans. The next day they were replaced in storage.

With the exception of slight temporary changes caused by opening the door, the storage room remained at a temperature of 17 ± 0.25° C. The relative humidity ranged from 67 to 70 per cent. of saturation.

Analytical methods

In the preparation of the bulbs for analyses, the dry scales, tips, and the dry parts of the bases were removed. The bulbs were cut longitudinally into two to six pieces, which were thoroughly mixed and ground in a meat chopper. As the dough-like mass coming from the machine could not be readily mixed or quartered it was returned to the hopper in small portions taken from different parts of the mash and reground. From the mass thus mixed, samples for analysis were weighed out. For moisture determination, samples of approximately 10 gm. were accurately weighed in weighing bottles and covered with absolute alcohol. After this had been evaporated at 60° C., the samples were dried at 70° and 16 mm. pressure in a slow current of dried air.

Twenty-five-gram samples for sugar determinations were washed into beakers with 100 ml. of 95 per cent. alcohol. Five-tenths of a gram of calcium carbonate was added to each sample. The beakers were heated to boiling for a few minutes and allowed to stand for 10 days, whereupon the alcohol was decanted into 250-ml. volumetric flasks. The residues were covered with absolute alcohol, and allowed to stand over night in a desiccator. The material thus hardened was ground in a mortar and extracted once by boiling with 70 per cent. alcohol, and for two periods of eight hours each in Soxhlet extractors.

This procedure was adopted after some preliminary experimentation during which it was found that interfering substances in the bulbs made the quantitative separation of the sugars difficult. The course of the sugar extraction in these preliminary experiments is given here. The percentages of the total extracted sugar found in the separate fractions of the extract were 58.98 in the storage alcohol; 39.90 in the filtrate from extraction by boiling; 0.60 in the first Soxhlet extract; 0.18 in the second Soxhlet extract; 0.06 in the third Soxhlet extract; 0.10 in the fourth Soxhlet extract; 0.07 in the fifth Soxhlet extract; 0.09 in a final extract made by boiling.

Each figure is the average of six determinations. In the regular work the extraction was terminated with the second Soxhlet extract.

The reductions were carried out essentially as described in a former paper (**7**).

Ten-gram samples for starch determinations were covered with 100 ml. of absolute alcohol and stored in flasks whose stoppers were sealed with paraffin. Ultimately the samples were ground in a mortar, transferred to porcelain extraction thimbles, and extracted with 500 ml. of cold 70 per cent. alcohol added in 10-ml. portions. Acid-hydrolyzable substances in the residues were determined by the acid-hydrolysis method of SACHSSE (**19**). This fraction is regarded as starch in the subsequent discussion.

All the data on carbohydrates were calculated in terms of grams of hexoses, in the equivalent at the different dates of sampling, of 100 grams of fresh bulbs at the beginning of storage. The calculation in case of each bag analyzed was based on the loss of weight of that particular bag from the day it was placed in storage until the day of analysis. Within the limits of the sampling error the figures are therefore all directly comparable.

For the purpose of finding the shrinkage of the bulbs during storage the bags were arbitrarily divided into two lots, the odd-numbered bags forming one lot and the even-numbered the other. The even-numbered lot contained the bags later to be treated with hot water. The shrinkage for each lot was determined separately, but in each case on a decreasing number of bags, since after each weighing two of each lot were used for analysis. The results showing the shrinkage on the basis of 100 gm. of fresh bulbs at the beginning are given in table I.

TABLE I

SHRINKAGE OF 100 GRAMS OF NARCISSUS BULBS AT DIFFERENT DATES DURING STORAGE

TREATMENT OF BULBS	JULY 11	JULY 25	AUGUST 8	AUGUST 22	SEPTEMBER 5	SEPTEMBER 19
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Untreated . .	100	96.97	95.07	93.49	92.51	91.34
Heat-treated Aug. 10	100	97.00	95.07	*93.22	*91.36	*89.33

* These figures represent the heat-treated lots.

It is evident from these figures that after the hot-water treatment the treated bulbs lose weight at a greater rate than the untreated bulbs, so that the difference in loss becomes greater with advance of the storage season. Although MÜLLER-THURGAU and SCHNEIDER-ORELLI (**13**) and IRAKLIANOFF (**9**) found that a temporary warming of dormant plant organs results in a passing increase in respiration, the greater loss in the treated bulbs can scarcely be attributed to this effect, for the excess loss of the treated bulbs over the untreated is about two grams for 100 grams of original fresh weight, while the difference in loss of total carbohydrates is only about three-tenths of a gram.

The greater loss in the treated bulbs is, therefore, chiefly due to greater loss of water. However, the moisture determinations given in table II show that there is no decrease in the moisture content of the living tissues of the bulbs during storage under the conditions of this experiment, but rather a slight increase caused by the accumulation of water of metabolism. The actual loss of water is apparently confined to the outer scales, which after exhaustion of their contents become brown and papery. It seems that the treated bulbs lose more water in this way than the untreated bulbs.

The starch changes occurring in the bulbs during storage are given in table III, which shows that there is a gradual decrease of starch during the entire storage season. After the hot-water treatment the loss from the

TABLE II

MOISTURE CONTENT OF NARCISSUS BULBS AT DIFFERENT PERIODS DURING STORAGE

	per cent.	*per cent.*	*per cent.*	*per cent.*
July 12	67.12	67.09	66.67	66.91
July 26	66.24	66.80	66.66	66.74
August 9	67.40	68.19	67.93	67.92
August 23	67.55	67.40	*67.89	*67.24
September 6	67.30	67.37	*67.52	*67.56
September 20	67.45	67.04	*67.19	*67.15

* These figures represent lots heat-treated Aug. 10.

treated bulbs is greater than that from the untreated. The loss of starch, in terms of dextrose, for 100 gm. of fresh bulbs at the beginning of storage, is 2.55 gm. for the untreated bulbs, and 3.15 gm. for those treated in the middle of the season. These figures are based on a comparison of the average of four samples at the beginning of storage with the average of two treated and two untreated, respectively, at the end of storage. The treated bulbs, therefore, lost for 100 gm. of original fresh material the equivalent in starch of six-tenths of a gram of dextrose more than the untreated.

TABLE III

STARCH, AS DEXTROSE, AT THE DIFFERENT SAMPLING DATES, IN THE EQUIVALENT OF 100 GRAMS OF ORIGINAL FRESH BULBS

	gm.	*gm.*	*gm.*	*gm.*
July 12	23.67	24.14	24.29	24.05
July 26	24.07	23.39	23.67	23.43
August 9	23.01	22.58	22.16	22.87
August 23	22.21	22.35	*21.74	*21.86
September 6	21.58	22.05	*20.91	*21.15
September 20	21.65	21.34	*20.85	*20.94

* These figures represent lots heat-treated Aug. 10.

The transformation of starch is first reflected in the increase of reducing sugars. The changes in these shown in table IV follow a very definite trend. During the first four weeks of storage, before any of the bulbs have been treated with hot water, there is a well-marked increase in reducing sugars. Thereafter, in the untreated bulbs the reducing sugars decrease until the end of the storage season, when the reducing-sugar content is scarcely above that of the bulbs at the beginning. In the treated bulbs, immediately after the hot-water treatment, the reducing-sugar content rises and remains for some time above that of the untreated bulbs, but finally falls back to the same level as in the untreated bulbs. This observation is

TABLE IV

Reducing sugar determined as invert sugar in the presence of sucrose, at the different sampling dates, in the equivalent of 100 grams of original fresh bulbs

	gm.	*gm.*	*gm.*	*gm.*
July 12	0.26	0.23	0.22	0.21
July 26	0.28	0.27	0.27	0.30
August 9	0.31	0.35	0.39	0.31
August 23	0.29	0.30	*0.36	*0.35
September 6	0.27	0.28	*0.31	*0.31
September 20	0.26	0.27	*0.25	*0.26

* These figures represent lots heat-treated Aug. 10.

in accord with the results of experiments of Müller-Thurgau and Schneider-Orelli (**13**), according to which hot-water treatment temporarily increases, under some conditions, the reducing-sugar content of *Convallaria* pips over those not treated.

Data in table V relating to sucrose changes show that increase in sucrose takes place chiefly during the last six weeks of storage coincident with the disappearance of reducing sugar. During the first four weeks there is no increase. After hot-water treatment the gain in the treated bulbs is greater than that in the untreated.

TABLE V

Sucrose, determined as invert sugar, at the different sampling dates, in the equivalent of 100 grams of original fresh bulbs

	gm.	*gm.*	*gm.*	*gm.*
July 12	3.11	3.05	3.19	3.12
July 26	3.09	3.08	3.17	3.13
August 9	3.13	3.02	3.03	3.10
August 23	3.27	3.26	*3.45	*3.59
September 6	3.47	3.54	*3.72	*3.74
September 20	3.54	3.55	*3.86	*3.86

* These figures represent lots heat-treated Aug. 10.

The carbohydrate changes in the bulbs stored under the conditions described are like those generally occurring in other starchy plant organs during dormancy. The essential feature of these changes is a gradual transformation of starch into sucrose. As is usually the case, at the relatively high temperature at which the narcissus bulbs were stored, the changes proceed slowly so that the total transformation is not great. The changes are accelerated in bulbs treated with hot water at some time during their summer storage. The effect of this more rapid transformation is not lost, but persists until the end of the season.

The possible influence of the accelerated changes in the treated bulbs on their subsequent growth may be considered as bearing a certain relation to the accumulation of sugar in plant parts as a result of treatments that break the rest period.

Experimental results differ as to the effect of an increase of sugar, in itself, in stimulating the growth of dormant organs, but it seems evident from PFEFFER's (**15**) critical analysis of the problem, as well as from the work of MÜLLER-THURGAU (**11, 12**) and of APPLEMAN (**1**) with potatoes, that both internal and external factors are concerned in inhibiting the growth of buds. According to MÜLLER-THURGAU's view growth begins as soon as the internal limiting factors, withholding the sugar supply from the buds, cease to exist. The direct injection of sugar has in many cases, but not always, been found to give favorable results. HOWARD (**8**) found that buds of *Crataegus macracantha, Fraxinus excelsior, Quercus pedunculata,* and *Q. rubra* showed growth when injected with distilled water or with sugar solution. In *Crataegus* and *Fraxinus* the effect of the sugar solution was somewhat greater than that of water. Buds of *Aesculus flava* showed no growth with sugar solution, but after 27 days gave slight growth without expanding of the leaves when injected with water. Increasing the sugar content of twigs by immersing them in a two or a four per cent. glucose solution or by injecting the solutions directly into the buds was found by BORESCH (**2**) to start the buds of *Corylus avellana, Tilia grandifolia,* and *Acer pseudoplatanus* into growth earlier than those similarly treated with water. He concludes that even a slight increase of glucose in the tissues is sufficient to bring about earlier growth. DENNY, (**4, 5**), however, found that, although reagents that were effective in breaking the rest period of potatoes also increased the sucrose content of the tubers, soaking the tubers in sucrose solution or injecting it into their tissues did not hasten sprouting. That treatments which promote growth are not always accompanied by an increase in the sugar content of the treated tissues is shown by the experiments of MÜLLER-THURGAU and SCHNEIDER-ORELLI (**14**) with *Convallaria* pips and with lilacs.

Since heat treatment of resting plant organs is very generally effective in starting them into growth, it may be assumed that the primary changes leading to growth are accelerated in narcissus bulbs by the hot-water treatment. The immediately apparent result of this treatment is an increased starch hydrolysis with a corresponding increment in sucrose which persists until the end of the storage season. At the time of planting, the treated bulbs contain more readily soluble carbohydrates than the untreated ones. This greater supply of immediately available reserve food may result in the better development of treated bulbs observed by growers, even though the stimulating effect of the hot-water treatment itself is not latently preserved, as MÜLLER-THURGAU and SCHNEIDER-ORELLI (**14**) have found with *Convallaria* pips stored subsequent to treatment. It is of interest to point out that the initial changes during storage are of the same general nature as those taking place during the subsequent growth of the plants, as shown by the investigations of CHRISTENSEN (**3**) and of MARIANNE PINKHOF (**16**) on the growth of tulips. That CHRISTENSEN could not correlate the extent to which these changes had proceeded before planting with the ease with which bulbs planted in October, December, and January, respectively, could be started into growth, scarcely bears on the question, since the bulbs at these widely separated dates could not have been otherwise comparable.

Summary

The changes taking place in narcissus bulbs stored at 17° C. may be briefly summarized as follows:

Untreated bulbs under the conditions of storage described in this paper lose about nine per cent. of their weight during a storage period of ten weeks. Bulbs heat-treated at about the middle of the storage season by immersion for four hours in water at 43.5° C. lose about eleven per cent. of their weight during the entire storage period. The greater loss of the treated bulbs probably arises from the fact that in these more of the outer scales become dry and brown than in the untreated bulbs.

Both in treated and in untreated bulbs there is a gradual loss of starch during the entire storage season. The loss becomes greater in the treated bulbs after treatment.

Reducing sugars increase gradually until about the middle of the storage season and then recede practically to the original quantity. The temporary increment is greater in the treated than in the untreated bulbs.

Sucrose undergoes scarcely any change during the first four weeks of storage, but thereafter increases until the end of the season. The sucrose content of the treated bulbs becomes greater than that of the untreated. The difference persists until the end of the storage period.

In the treated bulbs the metabolic processes are loosed or accelerated to a greater extent than in the untreated bulbs, so that at the time of planting the treated bulbs are not only more active in internal changes than the untreated bulbs, but also are supplied with a greater proportion of readily available carbohydrates. This state, it would seem, may enable the treated bulbs to get an earlier start or make a more active growth when planted.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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CATALASE ACTIVITY IN TOMATO FRUITS AT DIFFERENT STAGES OF THEIR DEVELOPMENT[1]

FELIX G. GUSTAFSON, INEZ CLARK, DANIEL A. SHAW
AND EDNA WARWEG

(WITH ONE FIGURE)

Ever since its discovery by LOEW catalase has been the cause of much speculation and investigation. During the thirty years since its discovery several hundred papers have been written concerning it, and we are still wondering whether its sole function is that of breaking down hydrogen peroxide into water and oxygen, or whether it has other yet unknown functions to perform. Because of the already profuse literature on the subject the briefest possible statements concerning the experiments performed will be given in this paper. Readers interested in the literature are referred to the paper by BATTELLI and STERN (2) in 1910 and one by MORGULIS (5) in 1924. Each of these has a very extensive bibliography.

For several years the senior author has been investigating the growth and respiration (3, 4) of tomato fruits. In this connection it was thought profitable also to make some catalase determinations on similar fruits, and find out whether there was any correlation between growth, respiration and catalase activity. Hence the catalase determinations here recorded.

The method of determining the catalase activity was the same as that employed by APPLEMAN (1). Fruits were sliced and placed in a saturated solution of $CaCO_3$ and left there a few minutes, and then ground in a food chopper. The juice was filtered through glass wool to free it from the pulp. One cc. of this juice was placed in a bottle (the reaction chamber) and one cc. of distilled water was added to this. The reaction chamber as well as the bottles containing the juice and the container of H_2O_2 were kept at 20° C. in a constant temperature bath, which did not vary more than half a degree either way. When the apparatus had been adjusted, 5 cc. of neutral H_2O_2 was added to the reaction chamber, which was then constantly shaken by hand. Readings of the oxygen liberated were taken every minute for five minutes. The total amount of oxygen liberated for the five minutes is used in calculations throughout this report. There was of course the usual dropping off of oxygen liberation as time went on.

The fruits used for these experiments were for the most part grown in the greenhouse, though a few were grown out-doors. The experiments were conducted at four different times by three different individuals, who beforehand were not familiar with the results obtained by the others. Thus the results have been checked several times. All determinations were made in duplicates.

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[1] Paper from the Department of Botany of the University of Michigan, no. 342.

By percentage of growth is meant the percentage increase in diameter per day. This was determined by measuring with a micrometer two diameters at right angles to one another and at right angle to the axis running through the fruiting stem. In most of the experiments the fruits were measured every three or four days for a long period of time, thus obtaining a growth curve. In a few of the later experiments, the fruits were measured only twice, twenty-four hours apart, the second measurement being made when the fruits were picked. GUSTAFSON (3) has shown that diameter

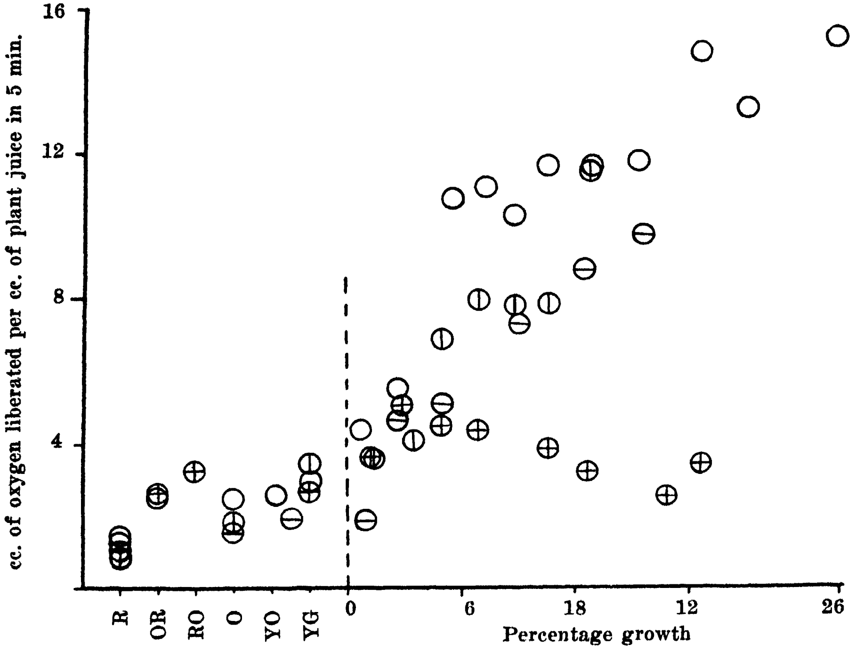

FIG. 1. This graph is plotted from the data given in tables I, II, III and IV. On the left of zero point are denoted different stages in ripeness of the fruits, while on the right of the zero are the percentages of growth the fruits made during the day preceding their picking. In designating ripeness a more or less arbitrary method has been used in noting colors: yg means yellow-green, yo yellow-orange, o orange, ro red-orange, or orange-red and r red-ripe. The figures are averages of the oxygen liberated in each group of experiments for the corresponding color or percentage of growth. The position of each point on the abscissa was determined by taking the average growth of all the fruits used in each group of experiments within ranges of every two per cent. The circles with a cross through them denote the data for the March–May, 1929, experiments, the plain circles data for the June–August, 1929, experiments, the circles with a perpendicular line through them the January–February, 1930, experiments and the circles with a horizontal line the May–June, 1930, experiments.

measurement is a perfectly satisfactory method of determining growth of a fruit. The John Baer tomato was used throughout this investigation.

The first experiments were conducted during March–May, 1929. During this period sixty-three determinations (in duplicate) were made in which sixty-nine fruits were used. The more rapidly growing fruits were quite small and several fruits were used in each determination. The data from this group of experiments are given in table I and figure 1.

TABLE I

Catalase activity determined March–May, 1929

Range (state of ripeness or percentage of growth)	Average growth in percentage within range	Number of experiments	Total number of fruits	Average amount of oxygen liberated in 5 minutes
	per cent.			*cc.*
Red		8	8	1.06
Orange-red		5	5	2.64
Red-orange		5	5	3.28
Yellow-green		7	7	2.73
0 – 2.0	1.13	12	12	3.65
2.1– 4.0	2.83	11	11	5.03
4.1– 6.0	4.0	4	4	4.56
6.1– 8.0	6.9	3	3	4.4
10.1–12.0	10.6	3	5	3.9
12.1–14.0	12.7	2	3	3.28
16.1–18.0	16.9	1	2	2.6
18.1–20.0	18.7	2	4	3.48

During the following June–August one hundred and seven more determinations were made in which one hundred and forty-eight fruits were used. Eleven of these experiments, using twenty-six fruits, were conducted with fruits grown out-doors. When fruits of the same growth rates are compared from the two sources there is no evident difference in the catalase activity. These determinations are given in table II and figure 1.

In January and February and again in May and June, 1930, more determinations were made. This time most of the determinations were made on rapidly growing fruits and only enough on the ripening and slow growing fruits to obtain an idea of the general behavior of these fruits. The first two groups of experiments gave different results for the rapidly growing fruits and it was thought very desirable to repeat this part of the experiments as extensively as possible. In the January–February lot, of the fifty-six fruits used thirty were growing at a rate greater than four per cent. and in the May–June lot, twenty-six out of thirty-eight were increasing at a rate greater than four per cent. for twenty-four hours, while in the first

TABLE II

CATALASE ACTIVITY DETERMINED JUNE–AUGUST, 1929

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		8	8	1.48
Orange-red		5	5	2.53
Orange		11	11	2.50
Orange-yellow		4	4	2.60
Yellow-green		13	13	3.00
0 – 2.0	0.7	19	19	4.41
2.1– 4.0	2.6	7	7	5.52
4.1– 6.0	5.7	5	5	10.81
6.1– 8.0	7.3	9	17	11.11
8.1–10.0	8.8	6	10	10.37
10.1–12.0	10.6	7	17	11.72
12.1–14.0	12.9	7	15	11.74
14.1–16.0	15.4	2	8	11.85
	18.8	1	3	14.85
19.1–22.0	21.2	2	3	13.27
	26.0	1	3	15.22

group of determinations only twenty-one out of sixty-nine grew that rapidly. The January–February and May–June determinations are given in tables III and IV as well as in figure 1.

TABLE III

CATALASE ACTIVITY DETERMINED JANUARY 31–FEBRUARY 10, 1930

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		3	3	0.86
Orange		3	3	1.85
Pink-green		3	3	3.46
0 – 2.0	1.4	7	11	3.6
2.1– 4.0	3.49	3	6	4.09
4.1– 6.0	4.91	3	6	6.95
6.1– 8.0	6.89	3	6	8.06
8.1–10.0	8.8	3	7	7.85
10.1–12.0	10.6	2	6	7.88
	12.8	1	5	11.6

TABLE IV

CATALASE ACTIVITY DETERMINED MAY 26–JUNE 4, 1930

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		2	2	1.35
Orange		2	2	1.53
Yellow		2	2	1.95
0 –2.0	0.95	1	1	1.90
2.1–4.0	2.62	2	5	4.67
4.1–6.0	5.15	2	5	5.15
	8.96	1	5	7.35
	12.54	1	8	8.82
	15.77	1	8	9.80

A glance at the graph shows that the amount of oxygen liberated by the fruits growing more rapidly than 4 per cent. per day was less than that liberated by those growing between 2 and 4 per cent. in the first group of experiments. In the last three groups of experiments there was a general, though irregular, increase in amount of oxygen liberated as the rate of growth increased. In other words as growth decreased catalase activity also became less. During the ripening process there seems to be a slightly lower activity in the yellow-orange and orange fruits than in the red-orange and red ones. If the graph, with the exception of the data from the first group of experiments for fruits growing more rapidly than 4 per cent., is compared with figure 3 in the paper by GUSTAFSON (4) on respiration of tomato fruits, one finds a general agreement between catalase activity and respiratory rate.

We have no explanation to offer for the divergence of the results for group one experiments with fruits growing over 4 per cent. Experiments have been conducted at all times of the year for two years. The fruits have come from plants both old and young, grown in the greenhouse and outside, yet only in this group of experiments did we get this low rate of catalase activity. Within themselves these aberrant determinations agree very closely, so it does actually seem as if they were not errors of sampling, as it would be rather hard to assume that the fruits collected over a period of nearly three months could all have been of a peculiar nature different from the normal fruits. Yet it seems very strange that we should not have run into these fruits at other times, but we never have. They are included in this paper even though they seem out of place, because we do not feel that experimental data should be suppressed merely because they do not fit in with the remainder.

In some of the fruits there is a slight indication that catalase activity is highest during the summer, but there is not enough evidence to be at all certain. From a few experiments a definite indication has been obtained that if two fruits having the same growth rate at the time of picking are used they will show a difference in catalase activity if one is still increasing its growth rate while the other is slowing down. The one that is on the ascending side of maximum growth has a higher catalase activity than the one that is past its maximum growth rate. From this one might argue that the catalase increase precedes increased growth rather than follows or accompanies it, and that catalase may in some way influence the rate of growth. This line of thought is however too dangerous to follow when based on as meager evidence as we have. It is much safer merely to state that respiration, growth and catalase activity go hand in hand and not try to assign any causal relation.

Our experiments then agree with those of some other investigators who have found that high catalase activity accompanies a high growth and respiratory rate.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN.

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EFFECT OF BORON ON THE GROWTH OF LETTUCE[1]

J. S. McHARGUE AND R. K. CALFEE

(WITH ONE FIGURE)

The results of a number of investigations on the influence of the less common elements on the growth of plants have shown boron to be essential for the normal growth and maturation of several varieties of green plants. A definite type of injury results in some species of plants from a deficiency of this element in the medium in which the plants are grown. The quantities required for normal growth have been reported as extremely small and a slight excess is toxic for those plants which have been tested. SOMMER and LIPMAN (**3**) concluded from their experiments that one-half part per million of boron is sufficient for the normal growth of most plants. They also presented evidence which shows that boron is necessary for the normal growth of a number of species of green plants. JOHNSTON and FISHER (**1**) found that boron in minute quantity is necessary for the growth and the fructification of the tomato plant, and that it is required throughout the growing period, an inadequate supply resulting in retarded growth, and in injury to the fruit. The investigations of SWANBACK (**4**) showed boron to be essential for the normal growth of tobacco, a deficiency of this element resulting in a stunting of the plants, and in serious injury to the growing points. Previous work by McHARGUE and SHEDD (**2**) showed that boron is essential for the growth of oats.

The effect on the growth of lettuce produced by a lack of boron was investigated by growing plants in a medium free from this element. The medium was prepared by mixing purified basal mineral nutrients with purified sand in the following proportions:

Purified sand (SiO_2)	1000.00	Gm.
Potassium dihydrogen phosphate (KH_2PO_4)	1.00	"
Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$)	0.75	"
Potassium nitrate (KNO_3)	0.5	"
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.5	"
Potassium chloride (KCl)	0.25	"
Potassium sulphate (K_2SO_4)	0.5	"
Hydrous ferric oxide ($Fe(OH)_3$)	1.0	"
Distilled water (H_2O)	200.0	"

Each of these chemical compounds was tested qualitatively and found to be free not only of boron, but also of manganese, copper, zinc, cobalt, nickel, barium, strontium, lithium, and arsenic. The sand was prepared by digesting it with hot hydrochloric acid in a large porcelain dish and then washing it free from chlorides. The distilled water used for the growth of the plants was condensed in a quartz tube. Acid-proof pots which had been previously washed with hydrochloric acid, autoclaved, and rinsed with distilled water were used in growing the plants.

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[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. Published by permission of the Director.

For experimental cultures, five kilograms of the purified basal medium was placed in each pot. The total weight was recorded and the moisture content kept at 20 per cent. through the growing period by the addition of distilled water until the required weight was reached.

Six pots containing six plants each of Big Boston head lettuce were included in the first experiment. A good germination of the seeds was obtained and a rapid growth was made by the young plants for the first two weeks. Growth during the third week was slower, and towards the latter part the newer leaves were noticeably lighter in color. The plants at this time had produced 10 to 12 additional leaves. At the beginning of the fourth week a few dark spots appeared on the tips of the younger leaves. These spots continued to increase in size and number, spreading along the margin of the leaves. All plants from which boron was withheld were affected in this manner. In a few days after the appearance of the spots the growing points died and turned black, giving the plants a badly burned appearance. The younger leaves continued to die, and in a few more weeks the entire plant was dead.

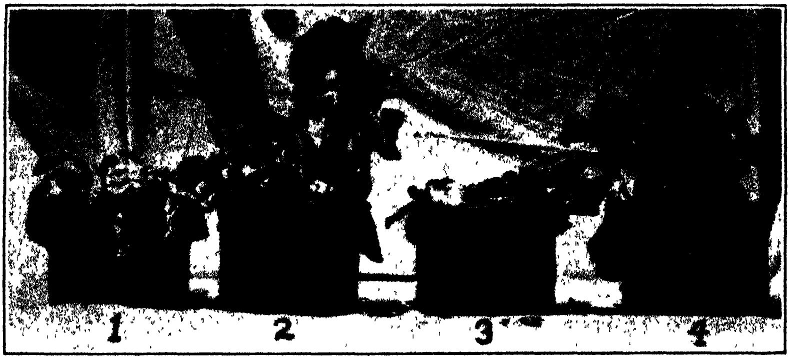

FIG. 1. The effect of boron on the growth of lettuce, other less common elements being present. Pots 1 and 3 contain no boron. The cultures in pots 2 and 4 received 0.0025 gm. of boron. Leaf lettuce, Black-Seeded Simpson, is in pots 1 and 2, and Big Boston head lettuce is in pots 3 and 4.

The presence of the disease at the same stage of growth in all plants indicated a nutritional deficiency rather than a disease of bacteriological etiology. Microscopical examination of the tissue failed to reveal any pathogenic organisms.

A second experiment consisted of negative control cultures, containing only the purified basal mineral nutrients; positive control cultures containing, besides the basal minerals, small amounts of manganese, copper, zinc, iodine, arsenic, and boron; and cultures containing only the basal minerals and boron. Big Boston head lettuce was again used as the experimental plant.

The growth of the negative control plants followed closely that of the plants in the first experiment. At the time the negative control plants developed the spots on their leaves, all plants receiving boron were normal and were considerably larger and greener in color. At the time all of the plants in the negative control cultures were dead, no plant receiving boron had developed any symptoms of any nutritional deficiency.

A third experiment with twelve pots of leaf lettuce (Black-Seeded Simpson) showed this plant also to develop the characteristic leaf injury when boron was absent. Treatments of various combinations of manganese, copper, zinc, nickel, cobalt, barium, strontium, iodine and arsenic failed to improve the condition. After the growing points of all plants were dead, a small amount of boron (0.005 gm.) in the form of calcium borate was added to each of six of the pots. Improvement was observed on the third day after this treatment. Dormant buds in the leaf axils started growth in all boron-treated cultures, and only in boron-treated cultures. The new leaves produced were normal in appearance. Rapid growth was made in all pots, and finally stalks which produced seeds developed. The plants in pots not treated with boron died, regardless of treatment with other elements. Other varieties of lettuce tested with similar results were Iceberg and Mammoth Black-Seeded Butter. These varieties, also, were found to burn unless boron was present.

Summary

1. Three varieties of head lettuce and one of leaf lettuce required boron for normal growth.

2. The deficiency of boron in cultures causes a burning of the leaves, and the death of the growing points of head and leaf lettuce at an early stage of development.

3. The function of boron in the metabolism of the lettuce plant is not performed by manganese, copper, zinc, nickel, cobalt, barium, strontium, iodine or arsenic.

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MOVEMENT OF ORGANIC MATERIALS IN PLANTS: A NOTE ON A RECENTLY SUGGESTED MECHANISM[1]

F. C. STEWARD AND J. H. PRIESTLEY

A recent paper by **CRAFTS** (1) discusses in an interesting manner the mechanism which effects rapid movement of organic solutes in the tissues external to the cambium. That such rapid movement, usually in a downward direction, does actually occur few will question. The general magnitude of sugar and also of organic nitrogen movement has been estimated by **MASON** and **MASKELL** to be of the order of physical diffusion in gaseous systems rather than the much lower rates, possible by diffusion alone in aqueous systems. Such a problem clearly demands careful scrutiny of any theory regarding a possible physical mechanism, especially one elaborated with due regard to the histological nature of the tissues concerned. **CRAFTS** suggests a mechanism very similar to that of **MÜNCH** (3) but is led by certain interesting anatomical observations to suggest that the translocation proceeds not in the sieve tubes (the most striking, longitudinally specialized cells of the phloem) but principally along the cell walls of the whole phloem tissues including sieve tubes, companion cells and parenchyma. The mechanism is not analogous to diffusion as **MASON** and **MASKELL** propose but is definitely dependent upon mass flow of solution in the cell walls. **CRAFTS** develops his theory as the result of a laudable attempt to decide anatomically the route offering least resistance to mass flow. The writers have every sympathy with such an anatomical approach but are equally clear that any apparent conclusions indicated on purely anatomical grounds must be interpreted strictly in accordance with physical principles and probability. We welcome the interesting contributions which **CRAFTS** has made to the histology of the phloem but it is the purpose of this note to show that some of the interpretations fundamental to the mechanism suggested are physically inadmissible.

CRAFTS' rejection of the sieve tubes as the path for translocation in favor of the cell walls is a distinctly novel feature. This follows principally upon the interesting observation that the total cross-sectional area of the phloem mounted fresh is composed of cell wall material in much greater degree than would be expected from preparations fixed and mounted in the usual manner. In the latter case it is shown that marked shrinkage of the wall occurs. The relative contributions of sieve tubes, pores in sieve plates, and the walls of all the phloem cells to the cross-sectional area of undried phloem were estimated by projection methods. **CRAFTS** arrives at the conclusion that, with due regard to the total area of sieve tubes, the presence of frequent sieve plates with small pores, and the hitherto unsuspected area of wall substance, the latter route would be the more efficient. This conclusion is justified on two principal grounds: (1) The calculated pressure gradient required to transmit a probable sugar solution by mass flow along the sieve tubes at a rate sufficient to ensure the observed transfer of dry matter (a) along a potato stolon, (b) in the stem of *Cucurbita,* (c) out of a leaf, are large (of the order of 20 atmospheres per meter). Similar calculations indicate that the corresponding pressure gradients necessary to effect transfer along the cell walls of the phloem are quite small (of the order of 1×10^{-6} or 6×10^{-5} atm. per cm. and actually as low as 1.6×10^{-8} atm. per cm. to secure transfer in a tree). (2) It is suggested that exudation studies on cut phloem tissues indicate normal mass flow in the walls of the phloem of an order quite adequate to secure the observed transfer of organic nutrients.

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[1] From the Department of Botany, University of Leeds, England.

The most important argument is that based on the required pressure gradients along the sieve tube and walls respectively. The necessary calculations were based on the familiar Poiseuille relation between the rate of flow and the pressure gradient along a tube of uniform cross-section, the viscosity of the solution, and the dimensions of the tube under conditions of uniform, non-turbulent flow. It is the purpose of the writers to show that the use made of the Poiseuille relation is such as to arrive at quite incorrect conclusions. CRAFTS arrives by a rather devious series of calculations at a figure for the pressure necessary to cause a mass flow at the required rate through the sieve pores of *Cucurbita.* In spite of the fact that the formula used is for uniform horizontal flow through a capillary this figure no doubt represents the order of pressure gradient required (20 atmospheres per metre). However, it is compared with a pressure calculated to represent that necessary to cause a mass flow at the required rate along the walls of the total phloem. It may be stated definitely that this pressure (1.5×10^{-5} atmospheres per cm.) has no relation whatsoever to the true pressure involved. The reason is fairly obvious. In this, as in all other cases where CRAFTS calculates pressure gradients necessary to cause mass flow along walls, he treats the total phloem wall area (or in the case of *Cucurbita* the wall area per bicollateral bundle) as a single pore. By various means this (the total wall area) was derived, and the radius of the equivalent circular area determined (see p. 18 and also p. 14, etc.). This radius was then inserted in the Poiseuille relation in which r can legitimately equal only the radius of a single capillary, thus:— $P = \frac{8Rnl}{\pi r^4}$.

where P = pressure in dynes per sq. cm.
R = rate of flow in cc. per sec. through total phloem walls.
n = viscosity of a 10 per cent. sugar solution.
l = length of gradient or tube in cm.
r = radius of conducting element due to the walls of which the shearing forces are exerted.
A = area of capillary through which flow takes place.
R_1 = linear rate of flow.

After CRAFTS:

$$R = R_1 \times A$$

$$P = \frac{8R_1 \times A \times n \times l}{\pi r^4}$$

$$P = \frac{8R_1 \times \pi r^2 \times n \times l}{\pi r^4}$$

$$P = \frac{8R_1 nl}{r^2}$$

where r = radius of circle equivalent to total phloem wall.

Correct:

$$R = R_1 \times A \times N \qquad A = \pi r_1^2$$

$$P = \frac{8R_1 \times A \times n \times l \times N}{\pi r_1^4}$$

$$P = \frac{8R_1 \times \pi r_1^2 \times n \times l \times N}{\pi r_1^4}$$

$$P = \frac{8R_1 \times N \times n \times l}{r_1^2}$$

where r_1 = radius of a single, circular, conducting element.
N = number of such capillaries in total area through which flow proceeds.

The resistance to flow through small pores depends on the shearing forces which are the result of steep velocity gradients in the moving liquid. The liquid in contact with the pore wall is theoretically at rest and that farthest from it has maximum velocity. In the Poiseuille expression for steady flow through a horizontal uniform circular capillary r represents the distance separating liquid surfaces at rest from that moving with maximum velocity. By utilizing in Poiseuille's expression the radius of the circular area equivalent to the total phloem area and a derived linear rate of flow, CRAFTS in effect says that the theoretical liquid surface at rest in contact with the pore wall is separated from that of maximum velocity by a relatively large length—the radius of the circle equivalent in area to the total phloem wall—whereas if flow did take place through the walls, it is clear that the true value should be of the order of half the mean wall thickness. In short, CRAFTS found the radius of the *actual pores* through the sieve plate, but when dealing with flow through the whole wall used a pore radius of a wrong order of magnitude. Since the formula demands $\frac{1}{r}$ to the fourth power it is clear that the derived pressures are of a totally wrong order of magnitude.

From the preceding formulae it is seen that one ought to multiply CRAFTS' derived pressures for mass flow through walls by a quantity $\frac{r^4}{r_1^4}$, since N obviously $= \frac{\pi r^2}{\pi r_1^2}$. This factor which equals the $\left(\frac{\text{radius of the equivalent single pore}}{\text{radius of the true, circular capillary pore}}\right)^4$ is large. If flow assumed through the whole wall is to be calculated as through circular pores at all, it would seem legitimate to state that r_1 at its maximum must be of the order of half the mean wall thickness. From Crafts' projection figures and magnification factors this can be estimated and r is known. It is thus possible to show that, whereas Crafts calculated that very small pressures would be adequate to cause the required mass flow along walls these are really of the general order of 10^6 times too small. For example, in the case of the potato stolon the value of 1.1×10^{-6} atmospheres per cm. is more probably of the order of 78.2 atmospheres per cm. Similarly the small pressure gradient of 1.5×10^{-5} atmospheres per cm. calculated as necessary to cause adequate mass flow through the walls of the phloem of *Cucurbita* becomes 11.89 atmospheres per cm. The same argument applies to all the pressure gradients calculated for flow through phloem walls. The bigger the area of the single pore used by Crafts (*i.e.*, the area of total phloem wall) the more improbable do his pressure gradients become. It will be noted that these more probable pressures assuming flow through the whole wall are either numerically greater than, or of the same order of magnitude as, those estimated to secure mass flow through the sieve pores. The conclusion, therefore, that the pressure gradients necessary to secure flow through walls are much less than those required to cause mass flow through the sieve pores, is on these grounds alone unsound. It is true that Crafts does not suggest that the total phloem wall is really a single pore, but he regards the factor of 4400 which represents the discrepancy between his derived pressure and that gradient which he conceives to be probable, as sufficient safeguard against the "walls acting as a single capillary and acting as they do in the plant"—(*l.c.*, p. 20).

It is clearly unsatisfactory to use the expression for flow through circular pores at all. The exact calculation for flow along a system of the type of the phloem wall would be difficult to compute. One can, however, estimate the pressure gradients necessary to cause the required mass flow along the phloem wall were this straightened out to form a single sheet bounded by parallel plane surfaces separated by the mean wall thickness. For uniform viscous flow of a liquid of viscosity (n) between parallel planes, the pressure gradient per cm. $\left(\frac{\partial p}{\partial y}\right)$ is given in terms of the volume rate of flow per sec. (V) across unit width (h sq. cm.) between planes h cm. apart by the formula (2) : $V = -\frac{h^3}{12n} \times \frac{\partial p}{\partial y}$ $V = Uh$ where U = linear rate of flow.

$$U = -\frac{h^2}{12n} \times \frac{\partial p}{\partial y}$$

For the case of *Cucurbita* (p. 18) using $U = 0.0759$ cm. per sec. (not 0.045 as Crafts[2] calculates)

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[2] Crafts derives the mean linear rate by dividing the mean of a series of total flows by the mean of a series of areas. He ought to work out the linear rates individually and find the mean of these. (See top, p. 16.)

$$h = \frac{0.25}{400}\text{cm. estimated from Plate Va}$$

$$n = 0.015$$

$$\frac{\partial p}{\partial y} = 3.50 \times 10^4 \text{ dynes per sq. cm. per cm. length}$$

$$= 0.035 \text{ atm. per cm. approximately.}$$

Pressure gradient per metre = 3.50 atm. approximately.

This value, which assumes in effect that the moving liquid is only sheared in a direction at right angles to the wall surface, is perhaps a minimum one and is of an entirely different order of magnitude from that calculated by CRAFTS. Further analysis of the application of the Poiseuille expression to mass flow of solution through the whole of the phloem walls seems superfluous since it is obvious that the whole wall cannot admit of mass flow. The actual pores are probably sub-microscopic and of the order of intermicellar spaces, *i.e.*, quantities to be measured in Ångström units. Without attempting further computation it is obvious that with this in mind even the preceding pressure gradients themselves of the order of 10^6 times those utilized by CRAFTS to support his thesis are really much too small. It is clear that CRAFTS himself realizes this point for in his discussion he says: "The fact remains that the spaces through which it is proposed that food materials in solution are passing are of molecular dimensions and irregular in form. Since no formulae are available by which to calculate pressure gradients and rates of flow through this type of material mathematical treatment is impossible. Use of Poiseuille's formula leads to high values fully as great as the ones which appeared on the sieve tube calculations."

In spite of this the whole case that the actual flow is through the walls depends for its justification upon the pores through which the flow takes place being not only much greater than the visible sieve plate pores but actually greater than the wall thickness itself. The probable pressures to secure mass flow through the whole wall are certainly either of the order of, or greater than, those calculated for flow through the sieve pores whose actual diameter was computed. From the molecular constitution of the cell wall as now known the presumption is that a mass flow could proceed more readily through the sieve tubes with their visible pores than along the phloem walls.

Since the application of the Poiseuille expression to the mass flow of solution along the phloem does not really justify the conclusion (as CRAFTS claimed) that the movement of organic solutes is along the walls, is there other evidence which does support such a novel idea? CRAFTS utilizes the well-known fact that the phloem of *Cucurbita* exudes droplets of sap as evidence of normally occurring mass flow in the phloem. From measurements of total phloem wall and also of sieve tube and sieve pore areas in the plants concerned he concludes by arguments identical with those already criticized that this flow takes place in the walls and not in the sieve tubes. We wish, however, to draw attention to the data which indicate that the rate of such flow, as determined by exudation studies, would be adequate to effect translocation into cucurbit fruits. The linear rate of flow presumed to be in the phloem walls was determined from the volumes collected in a given time. However, the flow was never observed for a period *longer than five minutes.* Where consecutive collections were made from one stem after a single cutting, the rate observed steadily decreased with time and stopped after five or six minutes (see table VII). In fact to make several collections from a single stem it was necessary to cut the stem repeatedly. This is ascribed to plugging of the cut surface by proteinaceous material which gelatinizes upon exposure to air. It seems much more probable that in a succulent plant of the type used the exudation from the phloem is not an indication of a normally occurring mass flow at all, either in the walls or sieve tubes, but may be due rather to the turgor of surrounding tissues and the release of tissue tensions consequent upon cutting. A new cut then merely opens up a new length of stem. However, apart from such a criticism, drastic as it is, CRAFTS is surely not warranted in comparing the rate of flow in cm. per min. observed over a period *not exceeding five minutes during which constant values were never attained* with the rate of flow calculated as necessary to account for uptake of dry matter by a pumpkin. In the latter case the rate is assumed uniform *over a period of 100 days* and the rate is calculated as cm. per min. Allowing such latitude in the choice of time intervals and such tolerance of what constitutes a steady flow can produce only purely fortuitous arithmetical agreement of results.

It is not our purpose to discuss the basic principle of the mechanism suggested by MÜNCH and CRAFTS, interesting as it is. We merely wish to point out that whether or not the phloem walls do form an important avenue for translocation, the data and argument used by CRAFTS to arrive at this conclusion are unsatisfactory, and ought logically to lead to a different one. The interesting fact remains that phloem *in vivo* has apparently much thicker, hydrated walls than we have hitherto supposed. That, however, does not necessarily indicate that they function in translocation over long distances. The application of formulae from classical physics, which apply usually to well defined cases which are rarely, if ever, satisfactorily duplicated in the living plant, involves often the adoption of arbitrary assumptions which may vitiate the conclusions drawn. We feel that in this particular case the utilization of the Poiseuille relation is particularly unfortunate and has led to entirely erroneous conclusions.

DEPARTMENT OF BOTANY,
UNIVERSITY OF LEEDS.

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BRIEF PAPERS

CONTAINER FOR THE STUDY OF THE BEHAVIOR OF INDIVIDUAL ROOTS[1]

(WITH ONE FIGURE)

In the course of studies on the water relations of pineapple plants it became necessary to measure the water absorption efficiency of individual roots. A container (fig. 1) was then constructed which served its purpose very well and also proved very useful for various other physiological studies. A description of the construction of this container and its operation is given so that other investigators interested in root studies may profit.

The construction of the container consists of four perforated detachable sides and a bottom, all of which are fastened to a framework either by means of screws or hooks. The container may be constructed either of wood or metal, the former being preferable for certain purposes and the latter for

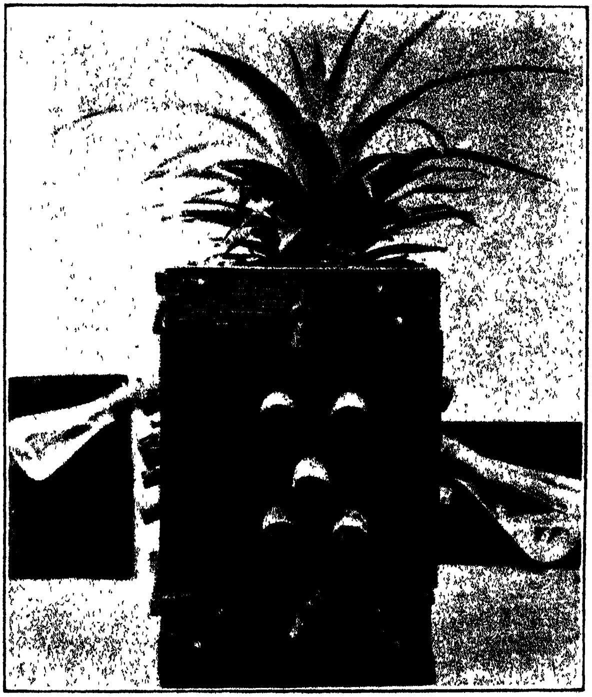

FIG. 1. Container for the study of the behavior of individual roots.

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[1] Miscellaneous Paper no. 9 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

others. It may be constructed in different sizes according to the size of the root system of the plants under experimentation.

The size of the container most suitable for our work measures 6 inches in width by 6 inches in depth and 8 inches in height. The substratum employed for the germination and subsequent growth of the plants in this container is river sand. After the plants have germinated and are growing the sides are opened at 2-day intervals and examined for roots. If such roots are in sight, proper connections are established by means of glass tubes between the root and outside container, a test tube or a flask, containing either a nutrient solution or soil. The glass tube, of an appropriate size, is connected to two rubber or cork stoppers, one of which is pointing towards the side of the container and the other one towards the flask containing the nutrient solution. The root is led into the glass tube very carefully and allowed to continue its elongation without any further disturbance. The vapor pressure of nutrient solution in the outside container is sufficient to maintain the root tip moist while in the glass tube and before reaching the solution proper. After the nutrient solution is reached, quantitative determinations of water absorption may be conducted. The results which one obtains by this method are very reliable, because there are no water losses other than those of absorption by the roots.

After the isolated roots become well established, further watering of the sand may be discontinued and the nutrition of the plant left entirely to the isolated roots. One can easily determine in this way not only the efficiency of individual roots for water absorption but also the number of roots which a plant must have at different periods of growth to carry on its normal physiological processes.—C. P. SIDERIS, *University of Hawaii, Honolulu, T. H.*

A SIMPLE DILATOMETER

(WITH ONE FIGURE)

The dilatometer here described was designed to measure accurately the changes in volume accompanying imbibition. The method has been used previously by **Hampton** (**1**), **Svedberg** (**2**), and others in studying the hydration of colloids. The dilatometer we have used in our work possesses certain advantages that facilitate its use and increase its accuracy.

The instrument consists of the usual bulb surmounted by a capillary tube in which volume changes are observed. There is an additional outlet with a stopcock for regulating the initial volume, and a mercury well or trap to keep the material being studied apart from the water until the beginning of the experiment. The dimensions given in the following description apply to the instrument we have used, but they may be modified as the materials or aims of the worker require.

As indicated in the accompanying figure the dilatometer consists of a heavy-walled glass bottle (500 ml.) fitted with a ground glass stopper. The lower half of the bottle is filled with some suitable cement except for a cylindrical well in the center. This well is conveniently formed by cementing a two inch piece of large bore (1–⅛″) glass tubing to the center of the bottom of the empty bottle and filling the space between the tubing and the bottle with cement. The cement is sloped upward from the rim of the well to the walls of the bottle. The cement used must be unaffected by water and must adhere firmly to the glass on cooling. We have found "Picein" satisfactory for this purpose, although other cements may be equally as good.

The ground glass stopper is drilled with two holes spaced opposite to each other just inside the flange of the stopper. A convenient drill for this purpose may be made by fastening a short piece of copper tubing of the desired diameter to the shaft of an electric motor with a piece of heavy-walled rubber pressure tubing. With ordinary valve grinding compound as an abrasive, such a drill will penetrate a thick glass stopper in a few minutes. A meter length of capillary tubing is inserted in one hole and a short stopcock tube is placed in the other. These tubes are held firmly in place by filling the hollow stopper with hot cement. Care must be taken to have the tubes and the cement level with the bottom rim of the stopper.

A lead weight (fig. 1, C) coated with cement is provided to hold the material beneath the mercury until the start of the experiment. Two parallel wires are cemented to the base of the weight to act as supports, and to hold it enough above the rim of the well to permit the introduction of mercury and the removal of the air during evacuation. A helical spring slightly smaller in diameter than the well is cemented by means of a short

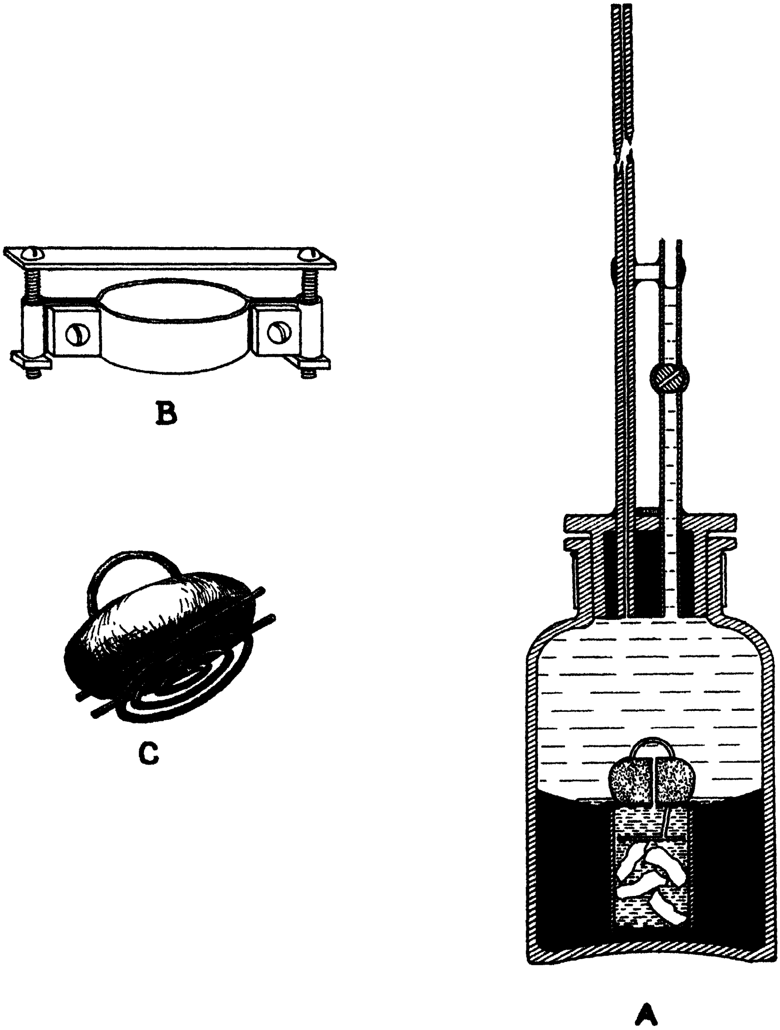

FIG. 1. A. Longitudinal section of the dilatometer. B. The clamp. C. The lead weight with spring.

wire to the base of the weight. This serves to hold the material beneath the mercury during the evacuation of the air and other preliminary operations. A small wire ring is fastened to the top of the weight to facilitate its manipulation in the bottle.

A clamp (fig. 1, B) is necessary to hold the stopper firmly in the neck of the bottle. This consists of a bar across the top of the stopper fastened with screws to a copper band around the neck of the bottle.

To operate the instrument the material being studied is placed in the well and the lead weight placed on top. Mercury is then added to more than fill the well and the bottle is evacuated in a chamber to remove all air from the mercury and material. After evacuation the bottle is filled to the top of the neck with distilled water that has been boiled to remove air. The water may be introduced as a fine stream through a glass nozzle by means of a siphon. If the glass nozzle is kept beneath the surface of the liquid, aeration of the water is avoided. The jet of water may be manipulated as the bottle is being filled to prevent the formation of air bubbles on the walls of the bottle or on the surface of the weight. When completely filled, the stopper is slipped into place in such a way as to force the water into both the capillary and stopcock tubes. The stopper must be firmly seated and clamped snugly in place. The bottle may again be evacuated through the capillary if desired, though for most purposes this is unnecessary. After adjusting the level of the water in the capillary tube to the desired height through the stopcock tube, the bottle is submerged in a constant temperature bath until equilibrium is attained. This is indicated by the constancy of the level of the meniscus in the capillary tube.

During these operations the colloidal material has been held beneath the mercury. To start the experiment the bottle is tilted slightly, allowing the weight to roll off the well and permitting the material to rise into the water. Readings of the height of the meniscus in the capillary taken at intervals will enable the operator to follow the progress of the hydration as closely as desired.

For use with powdered materials a small cup of glass, porcelain, wax or other suitable material may be used as a container. The cup is filled with the material which is held in place with a light plug of glass wool. The cup is then put into the well and the weight set in place as before.

We have found it desirable to run a simultaneous blank determination with water and mercury alone to permit corrections for minor changes in temperature. Because of the size of this particular dilatometer, variations of less than 0.1° C. in the temperature of the bath produced significant fluctuations in the level of the meniscus.

The instrument has proved to be satisfactory for research work requiring a high degree of accuracy and as a convenient method of demonstrating to classes the very considerable condensation of water accompanying imbibition.—D. B. ANDERSON, *Department of Botany*, and C. F. WILLIAMS, *Department of Horticulture, North Carolina State College, Raleigh, North Carolina.*

1. HAMPTON, H. C. A dilatometer for measuring the hydration of colloids. Science n.s. **63**: 49–51. 1926.
2. SVEDBERG, THE. Density and hydration in gelatin sols and gels. Jour. Amer. Chem. Soc. **46**: 2673–2676. 1924.

NOTES

New Orleans Meeting.—The eighth annual meeting of the American Society of Plant Physiologists at New Orleans was in certain ways the most important one held since the organization of the Society. The Executive Committee had considered many problems pertaining to the development and welfare of the Society and brought them before the annual meeting for action.

Included among these matters were the report of the committee which has been studying the advantages and disadvantages of incorporation; insertion of regulations for the life membership awards into the constitution; allocation of additional funds for the Secretary's office; and assistance in the editorial office. Other important matters have been pressing for consideration. More information in regard to the action taken at the annual meeting will be presented in the April number of PLANT PHYSIOLOGY.

Summer Meeting.—A summer meeting of the American Society of Plant Physiologists jointly with the Corn Belt Section of the American Society of Agronomy is being arranged, the meeting to be held at the University of Wisconsin, Madison, Wisconsin, probably in July, 1932. It is hoped that many of our members from the middle western states will keep this meeting in mind, and plan to attend it. The details of the arrangements will be included in the April number of PLANT PHYSIOLOGY.

The Sachs Anniversary.—The one hundredth anniversary of the birth of JULIUS VON SACHS, which falls on October 2, 1932, will be commemorated by botanists throughout the entire world. The American Society of Plant Physiologists has dedicated the 1932 volume of PLANT PHYSIOLOGY to SACHS in honor of this event. Steps are being taken to make the meeting at Atlantic City in December, 1932, a SACHS Anniversary meeting. We are happy to be able to present to our members the characteristic portrait of SACHS as a frontispiece to the volume. During the year an interior view of his famous laboratory, and other commemorative photographs will be reproduced, to revive and recreate memories of one of the greatest botanical leaders during the last half of the nineteenth century. His work has exerted a profound influence upon the development of botany during the last sixty-seven years, and will continue undoubtedly for many decades as one of the major forces in determining the trends of botanical research.

Membership.—During 1931 the publication of 1,000 volumes of PLANT PHYSIOLOGY cost practically the entire allotted income of the journal. The accumulated reserves and sale of back volumes will make possible some of the financial changes which the Executive Committee are recommending to the Society for adoption. Future increases in the size of the volumes published must be contingent, however, upon increased income. Fortunately the present income makes possible a very substantial volume. But the membership and subscription lists can be increased in numbers if each member will take a personal interest in offering the opportunity of membership to others whose work will be enriched and enlarged by possession of a personal copy of the journal for constant use. The Secretary will appreciate the assistance of all members in this enterprise of increasing the membership and library support of the Society.

Bibliography of Low Temperature Relations of Plants.—No good method of supporting the publication of bibliographies has as yet been found. Some time ago the Department of Agriculture of the University of Minnesota sent out an announcement of the preparation of an annotated bibliography of the low temperature relations of plants by Dr. R. B. HARVEY, who has spent much time in research in this field, and a great deal of time in accumulating the data for the bibliography. Publication, however, is contingent upon receipt of a sufficient number of advance subscriptions. In order to guarantee the costs of publication it will be necessary to secure about 500 subscriptions at $2.00 each. The estimated size of the volume is about 200 pages. It would probably be issued in stiff paper binding. Those who would like to help insure the publication of this volume can do so by writing to the Division of Publications, University Farm, St. Paul, Minnesota, and asking them to enter an order for one or more copies. Libraries might also be requested to order a copy conditional upon the receipt of a sufficient number of orders. Cooperation of physiologists in this way is about the only solution to the problem of publication of bibliographies.

Plant Physiology.—The second volume of Dr. S. KOSTYTSCHEW's Lehrbuch der Pflanzenphysiologie has been published by Julius Springer, Berlin. It is very fortunate that the work was completed while KOSTYTSCHEW still lived. The work will be his chief monument. This volume was shared with Dr. F. A. F. C. WENT, who wrote the chapters on growth and movement. The chapters by KOSTYTSCHEW are on the fundamental physiology of intake and translocation of materials, absorption and excretion, water relations of the plant, movement of plant sap in the vessels, and translocation and distribution of nutrients in the plant. The work taken as a whole is excellent, and deserves careful reading. The closing chapters by WENT are most difficult to handle. Plant physiologists in America will appreciate having these chapters written by the director of the Utrecht laboratory, where the most recent outstanding studies of plant movements have been made. No one engaged in research on the physiology of plants will want to omit this work from his ready reference library. It contains 459 pages, with 72 text figures. The price of the work bound in paper in 28 RM., and 29.8 RM. bound in cloth. Address Julius Springer, Linkstrasse, 23–24, Berlin W9, Germany.

Zonation.—A second edition of Dr. Ernst Küster's Über Zonenbildung in kolloidalen Medien has been published by Gustav Fischer, Jena. The first edition was published about 18 years ago, so the two editions differ greatly. The first chapter considers the phenomena described by Liesegang many years ago, which are often referred to as Liesegang rings. Chapter two deals with formation of sphaerocrystals and lamellated membranes. The third chapter takes up membrane thickenings, such as are found in the vascular elements, and lattice formation. The last three chapters are devoted to the color markings of leaves, flowers and seed coats, growth rings of dicotyledonous plants, and fairy rings of the fungi. The book thus brings together quite diverse phenomena which only by a great stretch of the imagination can be linked up with common causes. There is surely nothing in common between the annual growth rings of a tree and the true Liesegang periodic precipitation rings. Eight pages of literature citations and an index complete the volume, which contains 124 pages. The price of the volume in paper binding is 8 RM.

Experimental Plant Physiology.—A small book by Dr. G. J. Peirce, of Stanford, presents a running account of an experimental approach to the problems of plant physiology. While some directions are given for laboratory experiments, it is not a laboratory manual in the usual sense. It might serve as a basis for a well planned informal series of exercises, leaving much of the details of instruction with the person in charge of the class. The experiments are described in ten chapters, as follows: The properties of cells; absorption; the movement of water; the manufacture of food; the storage of foods; digestion; oxidative metabolism; growth; irritability; and reproduction. With index it contains 165 pages. The publishers are Henry Holt and Co., New York, who quote a price of $1.50 per copy.

Colloid Chemistry.—The third volume of Jerome Alexander's Colloid Chemistry has been published by the Chemical Catalog Co., New York. The papers included in this book make up the first series of papers on technological applications of colloid chemistry. There is to be a second series of these papers in the fourth and final volume of the set. Volume III contains 42 papers, most of which are of greater value to students of physical and chemical sciences than to biologists. The last four papers, however, are of interest in biology. Three of these deal with colloidal properties of soils, and the final one with the colloidal properties of wheat, wheat flour, and wheat flour products. The book contains 655 pages, and is priced at $10.50 by the publishers.

Introduction to Cytology.—Dr. L. W. SHARP's Introduction to Cytology has been revised and translated into German by ROBERT JARETZKY, Professor of Pharmacognosy in the Technical High School, Brunswick. Several hundred papers have been considered in the revision, and certain topics such as protoplasmic inheritance, conversion of genes, mitogenic rays, etc., have been given a place in the translation. There are 733 pages, including the index, and a literature list of 72 pages. The price of paper bound copies is 52.5 RM., and of cloth bound copies, 55 RM. Orders for this work should be sent to Gebrüder Borntraeger, Schöneberger Ufer 12a, Berlin W35, Germany.

A Shorter Course in Organic Chemistry.—Every plant physiologist is confronted with the necessity of keeping abreast of the developments in organic chemistry as a part of the background of plant physiology. A few books on organic chemistry must be kept at hand for consultation. Dr. J. C. COLBERT of the University of Oklahoma has prepared a foundation work which ought to be useful to those needing a short and elementary introduction to this fascinating field. It contains many useful diagrams, especially diagrams of the types of reactions which organic compounds can undergo. The emphasis is upon the understanding of fundamental theories, and the author has shown his pedagogical ability in the manner in which the material is presented. Copies may be had at $3.60 from the Century Co., 353 Fourth Ave., N. Y.

Julius von Sachs in seinem Arbeitszimmer Würzburg

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PHLOEM ANATOMY, EXUDATION, AND TRANSPORT OF ORGANIC NUTRIENTS IN CUCURBITS

ALDEN S. CRAFTS[1]

(WITH SIX PLATES AND ONE FIGURE)

Introduction

A survey of the subject of translocation of organic nutrients, in recent botany texts, indicates that mass flow through the sieve tubes, facilitated by perforation of the sieve plates, is the prevailing concept. Several authors distinguish between carbohydrates and proteinaceous materials as to their channels of movement, and some avoid the subject altogether. A majority favor this time-honored concept of the mechanism, however, although it has been seriously questioned in recent years.

Since the early descriptions of sieve tubes by HARTIG (**18**) and NÄGELI (**31**), the cucurbits have been widely used in the study of phloem anatomy. The clear preparations which may be obtained from these plants have undoubtedly been instrumental in the development of the sieve-tube theory of translocation. The ease of demonstrating phloem exudation in these plants is another factor which has strengthened the concept of mass flow through sieve tubes (**30**).

HARTIG described the sieve tube in 1837, gave an account of the sieve plate in *Cucurbita pepo* in 1854 (**18**), and described experiments with phloem exudates in 1858 (**19**). He considered the protoplasmic connections in the sieve plate to be uninterrupted, but VON MOHL (**29**) opposed this idea, holding that the pit-closing membrane is intact.

NÄGELI (**31**) gave an accurate description of the sieve plate; he undoubtedly observed callus formations; and he attributed a physiological significance to the characteristic slime accumulations so often observed in these tissues. He considered the sieve plate to be actually perforated, and thought that the slime accumulation was caused by a filtering of large particles from the moving sap. Under excess pressure the slime might even be forced through the pores according to his description. He proved that the exudate from these stems comes from the phloem, and used this as additional evidence for a mass flow through the sieve tubes.

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[1] National Research Council Fellow in Botany.

SACHS (**34**) advocated the idea that sieve tubes conduct only proteinaceous materials, carbohydrates moving through the remaining phloem elements.

The mechanism proposed over sixty years ago by these early workers is the one which is described in botanical text-books today. Subsequent ringing experiments have strengthened this view, while the occasional objections which have been proposed have been either overlooked or neglected.

The early works on sieve tube anatomy were collected by DE BARY (**2**), and later studies are well summarized by LECOMTE (**25**), PERROT (**32**), and HILL (**20**). It suffices to say here that they left unaltered the concept of mass flow in the sieve tubes. FISCHER (**14**) showed that the slime accumulations noted by NÄGELI were due to manipulation of the material, but it remained for LECOMTE (**25**) to give a proper interpretation of their formation. The latter work has been confined and extended and will be described in a later section. STRASBURGER (**41**) considered the sieve pores of angiosperms to be open, while those of certain gymnosperms are closed by median knots which he compared in permeability to the pit-closing membranes of bordered pits of xylem vessels.

CZAPEK (**8**) thought that all organic materials pass through the sieve tubes, but HABERLANDT (**16**) states, as did SACHS (**34**), that only nitrogenous compounds move in these elements. Physiologists still seem to favor the view that organic nutrients are conducted through the sieve tubes by a mass flow (**30, 36**) or by some sort of streaming movement (**6, 27**).

Anatomists (**2, 25, 40, 20**), while agreeing consistently that the protoplasm of the sieve tube is continuous through the sieve plate, have failed to question the existence of intervacuolar pores within the protoplasmic strands. They have considered that translocation and phloem exudation prove their existence. This attitude is well expressed by HILL (**20**), where on page 285 of his summary he says: "In the sieve-tubes there is the need of the formation of definite holes for the translocation of material, and the connecting threads serve as useful paths along which to work in order to produce tubes of sufficient diameter to enable an actual flow of slime to take place. Then, since the large holes produced are likely to be a source of danger to the plant when translocation diminishes the callus is utilized to regulate the bore of the tubes, and finally appears to put a stop to translocatory processes altogether." On page 262 of the same publication the sieve plates of *Vitis* are diagrammed, a clear distinction between slime and protoplasm being shown. In this author's own drawings such clear distinction is not shown, and in reading the text, the basis of differentiation is found to lie in the staining reactions of the connecting strands. In HILL's words (page 267), "The contents of these fine tubes are found to react in a different manner to stains after the appearance of the callus, for the protoplasmic threads of the embryonic condition have now become the more important slime strings of the mature sieve tube........"

More recent works on anatomy (**40, 11**) express a similar view on the structure of the protoplasmic connections of the sieve plate; in fact, the idea seems prevalent that these tubelike connections occur in most plants and that they provide adequate channels for longitudinal conduction.

There are two possible objections to this view, based on features of the sieve structure. If the middle lamella of the sieve plate actually closes the simple pits through which the protoplasmic connections pass (**29, 41, 25, 31**), mass flow would be seriously hindered. PERROT (**32**, p. 116) considered this of only secondary importance, and since the cellulose mesh of the plates of obliterated sieve tubes is actually perforated, it seems that at least during the more mature stages actual continuity through the pores is established. SCHMIDT's (**35**) work seems to confirm this, although the effect of the reagents used upon such a thin structure is somewhat doubtful.

The question as to the existence of intervacuolar connections or pores through the protoplasmic strands of the sieve plate is more serious. KUHLA (**24**) thought the connections to be solid and SCHMIDT's work indicates the same. The writer agrees with SCHMIDT on this point. In the first place, the actual protoplasmic strand is very narrow, approaching in most cases the limit of the resolving power of the microscope, so that differentiation is extremely difficult. In the second place, it is doubtful whether the reagents used will satisfactorily distinguish between protoplasm and vacuolar content in such minute strands.

Physiologists have apparently accepted the anatomists' view of sieve-tube structure until recently. RUHLAND's (**33**) studies on the permeability of sieve tubes aroused little comment, but the works of SCHMIDT (**35**), BIRCH-HIRSCHFELD (**4**), DIXON and BALL (**9**), DIXON (**10**), and CRAFTS (**5**) indicate from varying angles an increasing doubt as to the function of sieve tubes in translocation. The last paper, while giving little definite proof, strongly indicates that the sieve-tube mechanism is inadequate to explain longitudinal conduction in certain plants. The present paper takes up a more detailed study of sieve-tube anatomy in relation to exudation and translocation.

Phloem anatomy in cucurbits

It is evident that if a clear idea is to be obtained of the relationship of the different phloem elements to translocation, detailed studies of phloem anatomy are needed. This type of study precludes the use of many different species, and in the following work only two, *Cucurbita pepo* and *Cucumis sativus,* have been used.

In studying the phloem of cucurbits, it soon becomes evident that there are two distinct types of sieve tubes which, although having many features in common, can readily be differentiated on the basis of their structure and contents as well as on their location within the stem. In the transverse section of the stem of *Cucurbita pepo* diagrammed in text figure 1, the bicollateral vascular bundles and various sieve tubes are shown. FISCHER (**12, 13, 15**) has made a careful study of the sieve tubes of cucurbits and his nomenclature is used in this figure. In addition to the tubes of the vascular bundle, he distinguishes "ectocyclic tubes" lying between the epidermis and the sclerenchyma ring, "entocyclic tubes" lying inside this ring, and "commissural tubes," connecting the latter with those of the phloem groups of the bundle and connecting the peripheral tubes of the various bundle groups.

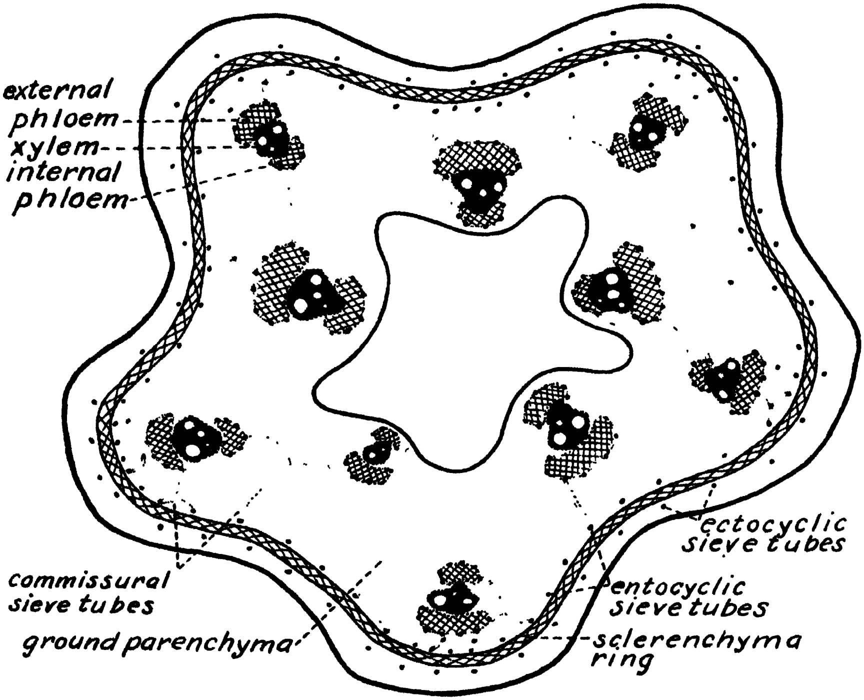

FIG. 1. Transverse section of pumpkin stem showing bicollateral bundles, ectocyclic and entocyclic sieve tubes. Commissural tubes follow curved and diagonal paths through the ground parenchyma. These paths as determined from serial sections are dotted in. Ectocyclic and entocyclic tubes are united only at nodes of stem.

These various tubes are shown in more detail in figures 1 and 2, and a distinction may clearly be made. The inner or central tubes of the phloem groups (fig. 1) are practically devoid of stainable contents, while the peripheral tubes of the groups (fig. 1) and the commissural and entocyclic tubes are densely filled.

On the basis of structure alone these various elements would fall into three groups: (1) the central tubes of the phloem groups of the vascular bundles, having short broad elements comparatively poor in stainable contents; (2) the longitudinal tubes of the periphery of the phloem groups, the ectocyclic tubes and the entocyclic tubes, all having on an average narrower and much longer elements and, in the mature state, densely staining contents; (3) the commissural tubes, varying greatly in length but resembling the second group in diameter, content, and sieve-plate structure, and connecting laterally the various longitudinally arranged tubes of the stem. Since the latter two groups are similar except in average length of elements and in position in the stem, two features which hardly allow for a physiological differentiation, they will be classed as one type in the following discourse, although the names given by FISCHER may occasionally be used.

The tubes lying in the center of the phloem groups will be mentioned first since they have been most widely described in the literature. The first distinguishing characteristics of the young differentiating sieve tubes in this material are the slime drops and the pitted end walls. The slime drops have been described and illustrated by FISCHER (**12**, fig. 9, Pl. I; fig. 4, Pl. II; fig. 9, Pl. IV; **15**, figures in Pls. I and II), LECOMTE (**25**, figs. 26 and 27, Pl. XXII), and others. They are translucent plastid-like bodies which lie in the parietal protoplasm of the developing cell. They can be distinguished during the early stages of vacuolation, before the cell has attained full size and while the nucleus is still prominent. At this time the protoplasm shows active streaming and the vacuoles will accumulate neutral red, but no protoplasmic connections can be distinguished traversing the end walls. It is interesting to note, however, that the middle lamella tends to stain deeply with neutral red, and by focusing carefully it can be seen that the portions occupied by sieve fields do not stain while the intervening portions do. This indicates that the sieve field regions differ in composition at an early stage.

In stems killed in hot water or 50 per cent. alcohol, the slime drops are fixed and stain readily with anilin blue (figs. 3, 4, 5). As the elements reach mature size, these structures enlarge and small vacuole-like spots become evident within them (figs. 6, 7, 8). At the same time the protoplasmic strands of the sieve field can be distinguished, and the first traces of the callus cylinders which eventually surround the strands appear (fig.

26). The nucleus is still present, but is larger and less deeply staining; the vacuoles unite to form a large central vacuole and protoplasmic streaming and neutral red accumulation diminish. As the elements reach maturity several rather complicated changes take place rapidly, making accurate description difficult. The sieve plate thickens, the original protoplasmic connections apparently fuse to form a single strand in each field, and the callus cylinders enlarge and elongate as described by HILL (**20**).

In the meantime that portion of the parietal protoplasm lying against the wall apparently increases in viscosity and ceases to stream, while anastomosing strands on the inner surface still show sluggish plastic movements. The slime drops enlarge further, the vacuoles within them increase in size and number, and stain is absorbed to a lesser degree in fixed material. The tendency for neutral red to accumulate in the vacuoles becomes much less pronounced at this time, although an accumulation by the vacuoles in the slime drops indicates that it is present in the protoplasm.

As the protoplasm becomes set on its outer surface, the inner strands tend more and more to push out from one slime drop to another across the vacuole (figs. 6, 7) and form an anastomosing network similar to that of the cells of epidermal hairs. An attempt has been made to illustrate this in serial order (figs. 8–12), but it is difficult since in fixed material the strands of the protoplasmic network within the vacuole shrink to mere threads and are not easily stained. This inner network of protoplasmic strands becomes fixed to the sieve plates of the end and side walls, loses its fluid consistency, and becomes relatively inert.

The existence of this inner structure in sieve tubes has been the subject of some controversy. FISCHER (**13**) disagreed with WILHELM's assumption of an inner sheath limiting the slime accumulation, or slime plug, considering it to be simply a slime formation. He later (**15**) described the contents of the sieve tube of cucurbits, making no mention of such an inner structure. ZIMMERMAN (**43**) seemed to find evidences of an inner structure which stained differently from the plasma sheath. He found it present in several species and in sieve tubes in different stages of maturity. The confusion which exists in relation to these inner threadlike structures can readily be understood, since any killing agent which causes a strong contraction of protoplasm will rupture them and collapse the outer sheath around the inclosed slime, completely obscuring them (fig. 19). An agent which kills very slowly, on the other hand, might cause a slow cessation of the plastic flow by which they are formed and so eliminate them before the cell is killed. In young living sieve tubes they are broad (fig. 6), tending to become more threadlike and numerous with maturity (figs. 7–14, 17, 18, 27, 28, 32, 33). They are seen only with difficulty in such tissues, and great care is required in preparation of sections as injury stops the flow of protoplasm in many cases. On the other hand, certain agents stimulate protoplasmic streaming (**37**), and this may explain the results obtained with low concentrations of alcohol as a killing agent in rendering these structures visible in fixed material.

The nucleus loses its staining properties as the elements mature, and apparently disintegrates, and neutral red is no longer accumulated within the vacuole. The slime drops, while tending to adhere to the protoplasmic strands of the vacuole, gradually swell, vacuolate, and disintegrate (**15**, p. 302), so that they become fewer in number and finally disappear as discrete bodies. The material resulting from disintegration of the nucleus and slime drops forms a colloidal suspension in the vacuole, and it is the coagulation and aggregation of this material which makes up the amorphous content of slime plugs in mature sieve tubes (figs. 27, 30).

The sieve plates assume their mature form, each protoplasmic strand being surrounded by a callus cylinder which projects beyond the level of the cellulose base. These cylinders may be seen (fig. 23) in transverse sections in which the knife has passed close enough to the plate to clear it of adhering protoplasm. By slightly raising the focus, the protoplasmic strand disappears and a light spot is seen in its place (fig. 24), due to an optical effect. The fact that DE BARY (**2**) mentions 5 μ for the maximum diameter for the sieve pores, while the largest protoplasmic connections measured in this work never exceeded 2 μ, indicates that the early workers possibly thought the callus cylinder to be the protoplasmic connection and the actual plasma strand to be a pore. This possibility is further indicated by the labeling of figure 65 *n* and 66 *l* on page 174 of the English translation of his book (**2**). Here he calls the structure comparable to the dark protoplasmic core in figure 23 an open pore. The sieve plate in figure 23 is shown in figure 25 with side illumination, bringing out even more the crater-like appearance of the ends of the callus cylinders which surround the protoplasmic connections.

In the development of definitive callus, these cylinders increase in diameter as they lengthen until they fuse laterally, forming a perforated plate (**15**) which increases in thickness, stretching the protoplasmic strands to many times their original length (fig. 31).

In all of the preparations which have been made in this work, there has not appeared a single indication of the presence of two phases within the protoplasmic connection of the sieve plate. The protoplasm changes in its staining reaction just as it does in its other physical properties. No evidence has been brought to light, however, which justifies the assumption of a "protoplasmic tubule" (HILL p. 261) and an inclosed "slime string."

Typical slime plugs occur only in the sieve tubes in the centers of the phloem groups. NÄGELI (**31**) observed these structures and proposed that they were the result of sap movement. FISCHER (**14**) found that by killing intact plants in hot water he could avoid their formation. He concluded that they were due to the rapid sap flow resulting from cutting the stem and thus steepening immensely the pressure gradient in the tissue. LECOMTE (**25**), however, found that slime plugs did not occur in fresh material but were the result of treatment with alcohol. This work has been repeated and confirmed, and the same results have been obtained by placing cut stems in hot water or by touching the end of a fresh section on a slide with a hot needle. These experiments indicate that slime-plug formation results from the action of agents which kill protoplasm and render it permeable. Following this action, turgor pressure or phloem exudation forces a part of the contents of the sieve-tube element through the permeable portion of the protoplast, filtering out any coagulated material.

Slime-plug formation is shown in sieve tubes in different stages of maturity in the series figures 11–16, the last being the appearance after the slime drops have almost completely disintegrated. In figure 29 the parietal protoplasm is shown in transverse section pulled slightly away from the walls. The slime plug shown in figure 28 is distinct and separate from this parietal protoplasmic layer, and at the lower end of the plug two strings of the inner meshwork may be seen. In figure 27 protoplasmic strings are shown traversing the total length of the cell. Three distinct strings can be seen emerging from the slime plug on the lower left side, while the parietal protoplasm remains but slightly removed from the walls. In figures 17 and 18 are shown two sieve tubes with slime plugs formed by killing with 50 per cent. alcohol. The parietal protoplasm remains practically intact and strings may be seen attached to the pits in the side walls. Killing with 95 per cent. alcohol results in more drastic shrinking, and the failure of past workers to distinguish between the parietal protoplasm and the internal meshwork is probably due to its use. In figure 19 the result of this protoplasmic shrinkage is shown, while in figure 20 the protoplasm has been torn completely free from the upper side of the plate, leaving small droplets where the strings have been broken.

By controlling the conditions, slime plugs may be formed on the two ends of a section pointing in opposite directions. When this is done, as by touching each end of a fresh section on the slide with a hot needle, the sieve tubes in the center of the section may show slime plugs in various positions (figs. 30, 32, 33, 36).

In the many stems used in this work, only one case was found in which slime accumulations could be seen in fresh material. This occurred in mature sieve tubes of a cucumber plant which was growing vertically on a string, and suggests that under certain conditions, after disintegration of the slime drops, suspended materials may gravitate to the lower ends of the cells as do starch grains in the sieve tubes of many plants. It may be said with certainty, however, that formation and orientation of the slime plugs have no correlation with the previous direction of sap flow in the plant.

The small mucous droplets or bubbles on sieve plates, mentioned by LECOMTE (**25**) as indicating the movement of sap through sieve tubes, are apparently as poor a criterion as are the slime plugs. NÄGELI (**31**) pictures these structures very clearly and they were often observed in the tissues being studied by the writer. They were never found very far from the cut end of the sectioned stem, however, and appear to be strictly localized in places where the pressure gradient has been immensely steepened by cutting. They also are found only in sieve tubes in certain stages of development, and appear to result from the semifluid protoplasm of the protoplasmic connection being forced through the pore and being inflated with sap within the vacuole of the succeeding sieve-tube element. The suggestion that it indicates transport of sap is difficult to understand, since its very structure shows that it would hinder rather than allow mass flow.

In a recent publication (**22**) JUNGERS proposed that the structures referred to in the literature as protoplasmic connections are in reality wall structures. Several of the preparations used in this work seem to refute this idea, although positive proof is difficult to produce. By carefully focusing on the structures pictured in figure 31, they can be followed without interruption from the protoplasm of one cell to that of the other. In figure 21 is shown a mature sieve tube in a stem killed by the use of hot water. The protoplasm contracted slightly, drawing out the protoplasmic connections without rupturing them, and they may readily be followed from the surface of the protoplasmic mass to the pore in the sieve plate. LECOMTE shows a similar preparation (**25**, Pl. XXI, fig. 8). In staining a section of epidermis from the vein on the back of a cucumber leaf to show protoplasmic connections, the protoplast was plasmolyzed, and in pulling the protoplasm away from the wall threadlike strands were left joining the connections in the pits of the wall with the external surface of the protoplast (fig. 22). These preparations seem to indicate the protoplasmic nature of the strands traversing the pit-closing membranes. If they are not protoplasm they are at least intimately connected with the protoplast, so that it should be very difficult on the basis of a microchemical test to prove that they have no protoplasmic content.

The second type of sieve tubes of the cucurbit stem has several distinctive features. While the elements of the central tubes of the phloem groups vary between wide limits in diameter and tend toward a uniformity in length, those of this type range from 0.05 to 1 mm. in length and approach a mean diameter of 0.02 mm.

In the early stages of development the two types are very similar in the nature of their contents (figs. 3, 34), although the slime drops tend to be smaller in the second type. As they approach maturity, however, distinct differences may be noted (fig. 35). The internal protoplasmic meshwork (figs. 27, 28, 32) is lacking, although a rather fibrous tuft may be seen on the sieve plate (fig. 35). The sieve plate fails to thicken as noticeably as in the internal tubes, and the vacuolar contents take on a granular appearance and become more and more dense (fig. 36). As the dense granular mass increases to fill the vacuole, a metamorphosis starts from the parietal protoplasmic layer and gradually approaches the center, the granular contents changing to an amorphous layer which eventually fills the tube completely. In figure 38 this change can be seen starting at the sieve plate, and in figure 39 a layer encircles the granular contents in the center tube while in the one on the right the contents have become completely amorphous. The sieve plate does not thicken during this development, but when the contents have reached the amorphous state, definitive callus is laid down (fig. 37). As the tube ages the jell-like content becomes broken by cracks and fissures (fig. 37) (**13**, Pl. IV, fig. 8; **15**, Pl. I, fig. 34).

Companion cells are very prominent with these sieve tubes (figs. 35, 39) and always accompany them. The commissural tubes (figs. 2, 40, 41) are much shorter in length than are those which traverse the stem longitudinally, but in other characteristics they resemble them closely. They are apparently cut from dividing ground parenchyma cells, and serve to link up the ectocyclic and entocyclic tubes with the peripheral tubes of the phloem groups as well as to connect the different phloem groups of the stem.

Slime plugs have not been found in this second type of sieve tube, although a density gradient in the granular contents in fixed material (**13**, Pl. I, fig. 6; **15**, Pl. II, fig. 44) indicates that a similar action occurs following treatment with killing agents.

Phloem exudation in cucurbit stems

HARTIG (**19**) described studies on phloem exudation in 1858. The exudate from cucurbit stems and fruits was analyzed by ZACHARIAS (**42**) and KRAUS (**23**), and was found to contain from 7 to 11 per cent. dry weight, of which a large portion was organic nutrient material. NÄGELI (**31**) thought that this phloem exudation from cut cucurbit stems proved the occurrence of a mass flow in sieve tubes. FISCHER (**14**), LECOMTE (**25**), HABERLANDT (**16**), MÜNCH (**30**), and many others have used the same reasoning in support of the sieve-tube theory of translocation.

In the course of the present work many measurements have been made on the rate of exudation from phloem tissues. In a previous paper (**5**) data were presented indicating an average rate of linear displacement of the total phloem volume of 0.8 cm. per minute for twenty collections, the maximum rate recorded being 3.5 cm. per minute. From another series of collections it was found that by successively cutting thin sections from the exuding end of a stem, exudation could be maintained for a considerable period of time. Several of the early collections, especially the one resulting in the high rate mentioned above, gave the impression that exudation was exceedingly rapid from young actively growing shoots. In order to carry this investigation further, a number of cucumber seedlings were grown in small pots and the young tender growing tips were dissected under a Greenough binocular. It was found that while exudation from the cut hypocotyl or elongated internode was comparable with that mentioned above, when the young tender tissues of the tip were severed an exceedingly high rate of flow resulted. The absolute amounts of exudate were not great, but in relation to the diameters of the structures cut, the spheres of exuding sap were large and developed with amazing rapidity. Tips of bracts, inclosing the young flowers, which were 0.5 mm. or less in diameter, would give rise to spheres of sap several times this diameter within a few seconds.

When a young leaf was cut the sap welled out along the entire cut surface, but when torn it could be seen in growing droplets upon the ends of broken veins. After removing several young leaves and flowers and allowing the escape of a relatively large amount of sap, new incisions would be followed by an apparently undiminished flow, as if the sap were coming from a large, elastic reservoir.

Histological examination of these young structures failed to reveal the presence of mature sieve tubes. In the younger bracts and leaves the phloem consisted of elongated parenchyma cells such as are described in bundle terminals by FISCHER (**13**) and STRASBURGER (**41**). The sieve tubes of the young stem were nearly all in the slime drop stage, and in fresh sections they accumulated neutral red. When plasmolyzed by killing in strong IKI solution, the protoplasm pulled away from the end walls as might be expected in ordinary elongated parenchyma cells.

Exudation from mature tissues proved to be most rapid from the peduncles of developing fruits of the cucumber, and rates will be given in the following section. Extending the observations on the period during which exudation could be maintained, several cucumbers weighing 200 gm. or more were cut from the plants, and by repeated cutting of the peduncles at intervals of about 15 minutes, continued exudation was induced. One fruit was still exuding after four hours of such treatment and another continued for two and one-half hours. When the fruits were inverted with their cut peduncles immersed in water, strings of coagulated exudate 6 inches or more in length could be seen hanging from the cut surfaces. When 50 per cent. alcohol was used, the coagulated exudate could readily be observed streaming down from the cut phloem groups.

Several full-sized cucumbers, having peduncles more than 1 cm. in length, were allowed to remain on the laboratory shelf for a period of six weeks, no precautions being taken to keep them cool or to prevent water loss. They were in diffuse light and the temperature averaged about 20° C. At the end of this period one of the fruits was examined for exudation by cutting off a piece of the peduncle about 3 mm. in length. The rate of exudation seemed normal, if not higher, so a collection was made on a second fruit, and the rate, given in detail in the next section, was found to be rather high. Upon sectioning the peduncles through which this exudation had occurred, it was found that all of the central sieve tubes of the phloem groups had large masses of definitive callus on their sieve plates, while the plates of the peripheral, commissural, and entocyclic tubes were free of callus with the exception of a few obliterated tubes. Tubes of the first type were practically free of stainable contents, while those of the second type were densely filled with a granular material, both in fresh and fixed sections. Masses of coagulated exudate adhering to the cut ends of the sections absorbed stain readily and had a colloidal structure which was practically amorphous, while the granules in the sieve tubes were similar in size to those shown in figures 34, 35, 38, and 39.

Green cucumbers hanging on plants which had matured and dried in the greenhouse were examined in a similar way, and, while exudation was still rather profuse the phloem proved to be in the condition just described. The central sieve tubes of the fruits, as well as those in the peduncles, proved to be callused.

In SCHUMACHER'S (36) recent work on translocation, he found the callusing of sieve plates to be a very sensitive reaction to immersion of leaves in eosin solutions. Finding the loss of nitrogen and dry weight from darkened leaves which had been so treated to be materially reduced, he concluded that the sieve tubes are the channels of transport for organic materials.

The effect of immersing cucumber leaves in eosin and in water, both in the light and dark, has been studied and the response described by SCHUMACHER has been confirmed. Practically all of the sieve plates in the main veins and petioles of the eosin-treated leaves were heavily callused within 48 hours, while immersion in water had no effect within that time. Leaves treated in the light reacted in the same way as those which were darkened, and cutting the veins was not essential to the response. The sieve plates of young sieve tubes in the slime-drop stage were callused as well as those of greater maturity. These young elements failed to accumulate neutral red when their plates were callused, and no protoplasmic streaming could be seen in them. Callusing occurred in sieve tubes of both of the types described in the previous section, and the accumulation of granules at one end of the cell in the cortical tubes was not hindered by its presence. Slime accumulations in the central tubes of the phloem groups were smaller or entirely lacking. After an exposure of 96 hours to the eosin solution, exudation was stopped, and microscopic examination showed that not only was callusing complete but the protoplasm and vacuolar contents were contracted and the walls collapsed, indicating that the cells had been killed.

Callus formation is typically a response to injury or degeneration of the sieve-tube elements. SCHUMACHER found it near the cut ends of stems, and in the present work it has occurred many times in stems collected in the greenhouse and placed in tap water in the laboratory. It generally occurs in old sieve tubes during their decline and is typical of the phloem of stored fruits. LECOMTE (**25**) described experiments in which he found that cucurbit seedlings grown in the dark had definitive callus upon the sieve plates of all mature sieve tubes.

SCHUMACHER comes to the conclusion that eosin is not carried back through the xylem, but that extension of the response for as far as 30 cm. from the point of application may result from movement of the eosin, or a stimulus resulting from eosin treatment, through the sieve tubes. Eosin is a very toxic substance, being even more harmful to plant tissue than arsenic. The fact that the mesophyll cells remain uninjured for several weeks after treatment suggests that the eosin did not enter them, yet it could enter the phloem only through them if it did not go through the xylem.

Dissection studies indicate an exudation pressure in the phloem and a subatmospheric pressure in the xylem. Since the solution covering the leaves in these treatments disappears more rapidly than can be accounted for by evaporation, it seems that it must enter the xylem and slowly displace the normal xylem sap, moving back down the petiole and into the stem. Since transpiration continues in the petiole, and uptake by the phloem is not entirely hindered, eosin would be carried into all of the surrounding tissues. In transverse sections of the petioles of leaves which had been immersed in a 1:25,000 eosin solution for 96 hours, the stain could be seen in the walls of xylem, phloem, cortex, and epidermis. Tests to be described later have given an indication that rather early in their development sieve tubes exhibit an increasing permeability with age as compared with companion cells and phloem parenchyma. This offers a possible explanation for their being so much more severely affected by eosin solutions.

Failure to obtain the typical response in abscissed leaves allowed to absorb eosin solution by transpiration might be due to rapid killing, resulting from accumulation of the stain in the tissues. When the treatment is applied by immersing abscissed leaves in the eosin solution, tension in the xylem is reduced as well as translocation in the phloem, so that injection of the stain could not be expected.

In the light of the experiments on exudation which have been described, it seems difficult to agree with the theory that definitive callus serves to control conduction by constricting the protoplasmic connections of the sieve plate (**16**, **20**, **25**, etc.), or that it may be of use to the plant in preventing the penetration of toxic substances (**36**). Since exudation is little affected by its presence or absence, the possibility of a relationship with perforation of the sieve plates seems even more remote.

The anastomosing system of sieve tubes which is found outside of the phloem groups of the vascular bundles resembles in several ways the open latex systems of many other plants. That it offered a ready explanation for the rapid flow of exudate seemed obvious, until a thorough study had shown that the end walls are typical sieve plates and that the parietal protoplasm persists throughout the life of these elements. The pores of the plates are very fine and are filled by protoplasmic strands. Thickening of the plate by callus is not so marked, and definitive callus is not formed until the contents have solidified, except from eosin treatment.

FISCHER (**13**) studied the development of this cortical sieve-tube system. stating that the elements were formed early in the development of the stem; that they functioned in the nutrition of the elongating tissues; and that obliteration occurred by the time the stem was fully mature. The writer's observations differ considerably from those of FISCHER. These tubes could be found fully developed in stems in various conditions from very young elongating internodes to peduncles of stored fruits. A few of them were found in the obliterated condition in stems, still undergoing elongation; yet if definitive callus on the sieve plate designates obliteration or a passive condition, as FISCHER assumes (**15**, p. 312), then these second type tubes were the only ones which were active in the peduncles of the stored fruits and senile stems mentioned above. On the other hand, if slime plug formation is a criterion of activity (**15**, p. 293), then even these were in a state of obliteration for they did not show these structures.

The most obvious conclusion which can be drawn from these various observations is that the criteria used by the early workers to designate the various stages in the development and function of these elements must be abandoned and a reinvestigation of the whole situation made, if we desire to know the true significance of sieve tubes in the physiology of the plant.

Experimental results

For a correct determination of the relation between phloem exudation and the transport of organic nutrients, quantitative data are required on the amounts of material being moved, the linear rates of flow, and the various factors affecting this movement. In a previous publication (5) data were given which indicated that materials were being translocated by this exudation process at somewhat higher rates than are required for growth and development of fruits of the pumpkin.

At the suggestion of Professor O. F. CURTIS, of Cornell University, the effects of local chilling were studied, since his experiments (7) had shown a definite retardation of translocation resulting from this treatment. Mature cucumber plants growing in pots were used, the treatments being carried on in the greenhouse. In the first experiment, four plants growing in a large pot, and trained to a height of about 4 feet, were employed and the equipment described by CURTIS and illustrated in text figures 1 and 2 of his publication (7) was used. The cooling coil consists of short lengths of rubber tubing fastened together by small glass U-tubes, a series of several lengths being bound around the stem and insulated from the air by covering with cotton.

In these experiments two stems were chilled and two were left unchilled. After collecting the exudate, the coils were removed and placed on the latter two plants and the previously chilled plants used as checks. This alternation was repeated several times and served to eliminate individual differences in the plants. In the first set one coil was used on each stem, covering a region about 2.5 inches in length. In making the collection, the stem was cut just above the coil and the cut basal end held in a small glass tube so that the exudate collected in the bottom. At the end of one minute the tube was removed, a thin slice cut from the end of the stem, and a second one-minute collection taken in the same tube. The tubes were stoppered and weighings made as soon as the samples were all taken. Two samples from chilled and two from unchilled plants were taken in one series. When all four were collected, a small portion of the end of each stem was cut off and taken to the laboratory for sectioning. These sections, mounted fresh in tap water, were placed in a microprojector and the enlarged image outlined on paper. The paper pattern was cut out and measurements made by weighing the areas and comparing with a standard area. The results are given in table I.

In order to test the effect of chilling upon the portion of the plant below the chilled region, sampling was continued on two plants, collections being made below the chilled region immediately following those taken in this region. This required sampling for 12 minutes, and two other plants, one normal and one chilled, were used to check upon the effect of such extended exudation. Table II summarizes the results obtained.

This experiment indicates that the effect of chilling is localized and that exudation from cuts made below the chilled portion of a stem are normal.

TABLE I

RATE OF FLOW OF EXUDATE FROM CHILLED AND NORMAL PLANTS

	Normal					Chilled			
Date	Time	Exudate fresh wt.	Phloem area	Linear rate	Average rate	Exudate fresh wt.	Phloem area	Linear rate	Average rate
	min.	*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*	*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct. 1	0–2	0.0256	0.508	2.52	1.77	0.0321	0.777	2.06	1.22
	2–4	0.0154		1.52		0.0137		0.88	
	4–6	0.0130		1.28		0.0110		0.71	
Oct. 2	0–2	0.0496	0.973	2.55	1.87	0.0199	0.557	1.79	1.20
	2–4	0.0342		1.76		0.0121		1.09	
	4–6	0.0256		1.31		0.0080		0.72	
Oct. 2	0–2	0.0432	0.858	2.52	1.65	0.0091	0.563	0.81	0.83
	2–4	0.0294		1.71		0.0100		0.89	
	4–6	0.0123		0.72		0.0088		0.78	
Oct. 2	0–2	0.0601	0.973	3.08	2.14	0.0113	0.383	1.47	1.13
	2–4	0.0378		1.94		0.0083		1.08	
	4–6	0.0271		1.39		0.0065		0.85	
Oct. 3	0–2	0.0436	1.088	2.00	1.56	0.0228	0.624	1.83	1.12
	2–4	0.0322		1.48		0.0111		0.89	
	4–6	0.0260		1.19		0.0082		0.66	
Oct. 4	0–2	0.0298	0.672	2.52	1.60	0.0278	1.246	1.11	0.76
	2–4	0.0191		1.42		0.0176		0.70	
	4–6	0.0158		1.17		0.0118		0.47	
Oct. 4	0–2	0.0325	0.592	2.74	1.81	0.0330	0.958	1.72	1.09
	2–4	0.0185		1.56		0.0178		0.93	
	4–6	0.0131		1.11		0.0121		0.63	
Grand average				1.79 ± 0.050				1.05 ± 0.047	
Difference				0.74 ± 0.07					

74 ÷ 1.79 × 100 = 41.3 per cent. decrease due to chilling. $\frac{D}{PE_D} = \frac{0.74}{0.07} = 10.6$

TABLE II

EFFECT OF LOCAL CHILLING UPON EXUDATION FROM UNCHILLED PORTION OF PLANT

DATE	TIME	TREATMENT	EXUDATE FRESH WT.	PHLOEM AREA	LINEAR RATE	AVERAGE RATE
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct. 3	0–2		0.0436		2.00	
	2–4	Normal plant	0.0322		1.48	1.56
	4–6		0.0260	1.088	1.19	
	6–8		0.0220		1.01	
	8–10		0.0192		0.88	0.91
	10–12		0.0182		0.83	
Oct. 3	0–2		0.0228		1.83	
	2–4	Chilled plant	0.0111		0.89	1.12
	4–6		0.0082	0.624	0.66	
	6–8		0.0081		0.65	
	8–10		0.0070		0.56	0.57
	10–12		0.0063		0.50	
Oct. 4	0–2	Collection from chilled portion	0.0278		1.11	
	2–4		0.0176	1.246	0.70	0.76
	4–6		0.0118		0.47	
	6–8	Collection below chilled portion	0.0336		1.48	
	8–10		0.0259	1.136	1.14	1.23
	10–12		0.0232		1.02	
Oct. 4	0–2	Collection from chilled portion	0.0330		1.72	
	2–4		0.0178	0.958	0.93	1.09
	4–6		0.0121		0.63	
	6–8	Collection below chilled portion	0.0378		1.61	
	8–10		0.0257	1.135	1.13	1.21
	10–12		0.0190		0.83	

During the foregoing experiments the temperature of the coils varied between 2° and 4° C., but the temperature of the greenhouse fluctuated between 20° and 30° C. Some samples were collected one morning when the temperature was 24° C. A second collection was made during the afternoon when it went down to 17° C. The results brought out the effect of cooling the whole plant as compared with local chilling and are given in table III. The three 2-minute collections are summed to save space.

These plants were mature and exudation from the tips was much more profuse than from the older stems, but upon this declining exudation rate the relative effects of cooling the whole plant as compared with local chilling are graphically impressed. With all four plants the lowest rate was measured at this time, indicating that a drop of 10° in the temperature of the total length of the conducting channels has a more profound effect than

TABLE III

EFFECT OF COOLING THE ENTIRE PLANT COMPARED WITH THAT OF LOCAL CHILLING

DATE	TIME	TREATMENT	TEMPERATURE OF GREENHOUSE CHILLING COILS		EXUDATE FRESH WEIGHT	PHLOEM AREA	LINEAR RATE
	min.				*gm.*	*sq. mm.*	*cm. per min.*
Oct. 20	0–6	Plant no. 1 chilled	20°–25°	1°–2°	0.0506	0.764	1.11
A.M. 21	0–6	" " normal	24°		0.0470	0.805	0.97
P.M. 21	0–6	" " chilled	17°	2°–3°	0.0140	0.761	0.23
A.M. 21	0–6	" " normal	26°		0.0452	0.980	0.77
Oct. 20	0–6	Plant no. 2 chilled	20°–25°	1°–2°	0.0487	1.067	0.76
A.M. 21	0–6	" " normal	24°		0.0744	0.914	1.36
P.M. 21	0–6	" " chilled	17°	2°–3°	0.0272	0.845	0.54
A.M. 22	0–6	" " normal	26°		0.0661	0.825	1.34
Oct. 20	0–6	Plant no. 3 normal	20°–25°		0.0653	0.672	1.62
A.M. 21	0–6	" " chilled	24°	3°–4°	0.0347	0.645	0.89
P.M. 21	0–6	" " normal	17°		0.0056	0.702	0.13
A.M. 22	0–6	" " normal	26°		0.0522	0.821	1.06
Oct. 20	0–6	Plant no. 4 normal	20°–25°		0.0499	0.814	1.02
A.M. 21	0–6	" " chilled	24°	3°–4°	0.0224	0.726	0.51
P.M. 21	0–6	" " normal	17°		0.0100	0.662	0.25
A.M. 22	0–6	" " normal	26°		0.0311	0.786	0.66

chilling a much shorter region. This result is to be expected, since in viscous flow the resistance is directly proportional to the length of the conducting element.

Realizing the seriousness of fluctuating external temperature upon exudation rates, the experiment reported in table I was repeated, an attempt being made to maintain the greenhouse temperature at 20° C. or slightly above. In this set, two chilling coils were used on each stem covering a region about 5 inches in length. At the time the collections were made the upper coil was removed and the initial cut was made in the center of the region which it covered. The results are summarized in table IV.

These results show definitely that localized chilling hinders exudation, although with the length of stems used it was not stopped. CURTIS found with his bean plants that chilling the petioles "stopped or greatly retarded" the removal of carbohydrates (7). With the type of treatment used here, exudation was retarded only about 50 per cent. There are two possible reasons, however, for this difference in results. In the first place he chilled a much greater proportion of the total stem length of his plants, and since viscous flow is hindered in direct proportion to the length of the conducting

TABLE IV

Rate of flow of exudate from chilled and normal plants; chilling coil temperature, 2°–4° C.

		Normal					Chilled				
Date	Time	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate	Plant number Greenhouse temperature	Fresh weight	Phloem area	Linear rate	Average rate
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*		*gm.*	*sq. mm.*	*cm. per min.*	*cm. pr. min.*
Oct. 22	0–2	7	0.0452	0.571	3.98	2.62	5	0.0150	0.488	1.53	0.81
	2–4		0.0241		2.12			0.0061		0.62	
	4–6	22°	0.0203		1.79		22°	0.0026		0.26	
Oct. 22	0–2	8	0.0438	0.775	2.83	2.28	6	0.0168	0.674	1.24	0.64
	2–4		0.0336		2.17			0.0066		0.49	
	4–6	22°	0.0285		1.84		22°	0.0023		0.17	
Oct. 23	0–2	5	0.0354	0.788	2.24	1.48	7	0.0180	0.530	1.69	1.18
	2–4		0.0203		1.29			0.0113		1.07	
	4–6	23°	0.0143		0.91		23°	0.0084		0.79	
Oct. 23	0–2	6	0.0195	0.726	1.34	0.99	8	0.0228	0.906	1.26	0.94
	2–4		0.0117		0.81			0.0166		0.92	
	4–6	23°	0.0119		0.82		23°	0.0115		0.63	
Oct. 24 A.M.	0–2	7	0.0315	0.752	2.09	1.47	5	0.0153	0.952	0.80	0.49
	2–4		0.0191		1.27			0.0059		0.31	
	4–6	24°	0.0155		1.03		24°	0.0066		0.35	
Oct. 24 A.M.	0–2	8	0.0403	1.101	1.83	1.41	6	0.0129	0.913	0.71	0.54
	2–4		0.0264		1.20			0.0077		0.42	
	4–6	24°	0.0266		1.21		24°	0.0088		0.48	

TABLE IV.—(*Continued*)

		NORMAL					CHILLED				
DATE	TIME	PLANT NUMBER GREENHOUSE TEMPERATURE	FRESH WEIGHT	PHLOEM AREA	LINEAR RATE	AVERAGE RATE	PLANT NUMBER GREENHOUSE TEMPERATURE	FRESH WEIGHT	PHLOEM AREA	LINEAR RATE	AVERAGE RATE
	min.		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*		*gm.*	*sq. mm.*	*cm. per min.*	*cm. per min.*
Oct. 24 P.M.	0–2	5	0.0244	0.921	1.34	0.92	7	0.0137	0.704	0.97	0.74
	2–4		0.0146		0.80			0.0104		0.74	
	4–6	24°	0.0118		0.65		24°	0.0071		0.50	
Oct. 24 P.M.	0–2	6	0.0238	1.054	1.34	1.10	8	0.0227	1.454	0.78	0.58
	2–4		0.0199		0.94			0.0114		0.39	
	4–6	24°	0.0217		1.03		24°	0.0162		0.56	
Oct. 25 A.M.	0–2	7	0.0386	0.840	2.30	1.57	5	0.0126	0.977	0.65	0.45
	2–4		0.0240		1.43			0.0080		0.41	
	4–6	21°	0.0167		0.99		21°	0.0059		0.30	
Oct. 25 A.M.	0–2	8	0.0517	1.673	1.55	1.03	6	0.0071	1.115	0.31	0.22
	2–4		0.0303		0.91			0.0043		0.19	
	4–6	21°	0.0215		0.64		21°	0.0034		0.15	
Grand average						1.49 ± 0.12					0.66 ± 0.06

Difference = 1.49 − 0.66 = 0.83 ± 0.13.

0.83 ÷ 1.49 × 100 = 55.7 per cent. decrease due to chilling.

$$\frac{D}{PE_D} = 6.38.$$

element and also in direct proportion to the viscosity of the solution, greater retardation was to be expected. In the second place he was measuring normal flow while in this case a rate of three or more times that of normal transport was being dealt with. The resistance in a closed system might be great enough practically to stop the flow, but if the length of the pressure gradient were suddenly shortened by cutting, rapid flow would occur until equilibrium was reached.

It seems justifiable, in the light of these experiments, to conclude that the effect of local chilling upon exudation is due to the change in viscosity of the solution of nutrients; and while protoplasmic streaming may be stopped owing to a similar increase in viscosity, it does not necessarily follow that a more direct relationship exists between protoplasmic streaming and longitudinal transport of solutes.

In order to see whether local chilling had any effect upon the composition of the exudate, some of the samples were dried to constant weight and the dry weights calculated as percentage of fresh weight. Table V shows that no significant difference exists between these samples.

TABLE V

DRY WEIGHT OF EXUDATE FROM CUCUMBER STEMS

		NORMAL			CHILLED		
		TIME (MIN.)			TIME (MIN.)		
DATE	STEM	0–2	2–4	4–6	0–2	2–4	4–6
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Sept. 30	1	8.6	7.6	6.6	10.5	9.1	7.5
	2	8.3	6.9	5.8	9.0	7.9	9.3
	3	10.6	11.0	7.8	8.8	7.5	6.5
Oct. 1	4	8.8	6.4	7.5	8.5	6.4	6.0
Average		9.1	8.0	7.0	9.2	7.8	7.4

The next set of experiments was designed to show the relation between the composition of the exudate, the composition of the fruits, and the rate of development of the fruits. On October 3, several young fruits were tagged and two were collected, the exudate from the stem attached to the plant being taken at the same time. The results are shown in table VI.

The marked cucumbers were collected on October 9, 15, and 30. The average weights on the two small ones are included in table VII to give the complete series.

TABLE VI

FRESH WEIGHT AND DRY WEIGHT OF FRUITS AND EXUDATE FROM CUCUMBER PLANT; EXUDATION RATE FROM PLANT

DATE AND PLANT	TIME	EXUDATE						FRUIT		
		FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE	AVERAGE RATE	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH
	min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
Oct. 3, Plant no. 3	0–2	0.0535	0.0051	9.5	0.617	4.33	3.72	0.930	0.0693	7.45
	2–4	0.0500	0.0037	7.4		4.05				
	4–6	0.0341	0.0021	6.2		2.76				
Oct. 3, Plant no. 4	0–2	0.0527	0.0044	8.4	0.567	4.65	3.19	0.789	0.0623	7.90
	2–4	0.0314	0.0022	7.0		2.74				
	4–6	0.0246	0.0013	5.3		2.17				

TABLE VII

Composition of cucumbers and increase in weight with development; exudate from plant

Date	Time	Exudate					Fruit		
		Fresh weight	Dry weight	Dry percentage of fresh	Phloem area	Linear rate	Fresh weight	Dry weight	Dry percentage fresh
	min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
Oct. 3	0–2	0.0531	0.0043	8.1	0.592	4.50	0.86	0.066	7.7
" 9	0–2	0.0597	0.0046	7.7	0.540	5.50	8.42	0.453	5.4
" 15 . . .	0–2	0.0813	0.0053	6.5	0.964	4.30	72.00	2.610	3.6
" 30 . . .	0–2	0.0859	0.0053	6.2	1.395	3.07	138.50	5.740	4.1

It is evident that the exudate is consistently higher in dry weight than the fruits, and that the difference increases with development of the fruits.

Two interesting calculations may be made from these data. Between October 9 and 15 the fruits gained at a rate of $2.610 - 0.453 \div 6 = 0.36$ gm. per day in dry weight. The sap at this time had a dry weight percentage of 6.5. Then $0.36 \div 0.065 = 5.5$ gm. of fresh sap required daily. But $72.00 - 8.42 \div 6 = 10.6$ gm. daily increase in fresh weight. The fruit, therefore, was requiring 5.1 gm. of water daily in order to maintain growth. Dividing 5.5 by 0.00964 cm.^{2} we find a rate of flow of 570.5 cm. per day, or 0.396 cm. per minute for the sap. But the recorded average rate of exudation is 4.50 cm. per minute, or approximately eleven times the normal rate of flow.

If a similar calculation is made for the rate of flow of sap required to develop the full-sized cucumber, a value is obtained which approximates very closely that of 0.292 cm. per minute previously obtained for the pumpkin (5). $5.740 - 0.066 \div 0.065 \div .00964 \div (24 \times 60) = 0.235$ cm. per minute average rate of flow.

TABLE VIII

FRESH AND DRY WEIGHTS ON FRUITS AND EXUDATES OF CUCUMBER PLANTS; SAP COLLECTED FROM PLANT END

No.	SAP					FRUIT		
	FRESH WEIGHT	DRY WEIGHT	DRY PER-CENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE	FRESH WEIGHT	DRY WEIGHT	DRY PER-CENTAGE FRESH
	gm.	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
1	0.1033	0.0081	7.8	0.860	6.00	251.6	9.72	3.9
2	0.1020	0.0080	7.8	1.100	4.63	121.6	5.24	4.3
3	0.1390	0.0116	8.4	1.180	5.88	121.7	4.91	4.0
4	0.0877	0.0067	7.6	0.936	4.69	187.4	7.20	3.9
5	0.0843	0.0066	7.8	0.692	6.10	93.0	3.45	3.7
6	0.0800	0.0052	6.5	1.100	3.64	116.0	3.97	3.4
7	0.1125	0.0090	8.0	1.225	4.59	183.5	7.15	3.9
8	0.0945	0.0071	7.5	0.548	8.62	87.5	3.15	3.6
9	0.0813	0.0053	6.5	0.964	4.23	72.0	2.61	3.6
10	0.0763	0.0061	8.0	0.926	4.12	35.5	1.57	4.4
Total	0.9609	0.0737	Av. 7.7	9.531	Av. 5.05	1269.8	48.97	Av. 3.9

On October 15 a series of ten fruits, ranging from half-grown to full-grown, was collected and their fresh and dry weights along with similar data on the exudate were obtained (table VIII). The exudate was collected for two successive one-minute periods. The figures of this table show that the cucumber maintains about the same dry weight composition during the later period of its enlargement, and that this is approximately one-half that of the sap exuded from the cut peduncle. The rate of exudation is extremely high, indicating low resistance to flow into the fruit.

This study was carried further by making dry weight determinations on fruits, stems, leaves, and on the exudate from the stem and fruit (table IX).

TABLE IX

DRY WEIGHT COMPOSITION OF CUCUMBER PLANT

WEIGHT	STEM		PETIOLES	LEAVES	FRUIT	EXUDATE	
	ABOVE FRUIT	BELOW FRUIT				FRUIT END	STEM END
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Fresh	4.0520	5.5338	4.7839	7.7118	127.50	0.0341	0.0285
Dry	0.3518	0.5221	0.3648	1.0835	4.9750	0.0041	0.0031
Dry percentage of fresh	8.67	9.42	7.64	14.10	3.90	12.00	10.90

The exudate from the peduncle of the fruit was collected for a period of 16 minutes and fresh and dry weights determined. Cross-sections were made of the initial and final areas of the peduncle, and areas interpolated between these values used to determine exudation rates (table X). A thin section was cut from the end of the peduncle each minute during the collection.

It will be seen from the data of table X that there is no great difference between the rate of exudation and dry weight composition of the exudate from the fruit and from the plant. The decrease in rate and dry weight of the exudate with time is to be expected, and points to an osmotic system regaining equilibrium after being thrown out of balance by suddenly releasing the internal pressure.

Data were collected on the stored fruits previously mentioned and are given in table XI.

Exudation from these stored fruits was not maintained as in the fresh ones. The xylem was full of tyloses and had lost the water from many of its larger vessels, so that the main source of the exudate was the more or

TABLE X

RATE AND COMPOSITION OF EXUDATE FROM CUCUMBER STEM AND FRUIT

	STEM	FRUIT										
Time (min.)	0–1	0–1	1–2	2–3	3–4	4–5	5–6	6–8	8–10	10–12	12–14	14–16
Fresh weight	0.0285	0.0341	0.0393	0.0341	0.0331	0.0302	0.0215	0.0369	0.0280	0.0314	0.0343	0.0239
Dry weight.....	0.0031	0.0041	0.0045	0.0038	0.0033	0.0027	0.0021	0.0025	0.0018	0.0018	0.0017	0.0010
Dry percentage of fresh	10.9	12.0	11.4	11.1	9.9	9.0	9.8	6.8	6.4	5.7	5.0	4.2
Phloem area, sq. mm.	0.727	0.727	0.800	0.868	0.937	1.005	1.072	1.205	1.341	1.466	1.610	1.738
Linear rate, cm. per min.	3.92	4.70	4.90	3.92	3.45	3.00	2.00	1.53	1.04	1.07	1.06	0.69

TABLE XI

DRY WEIGHT COMPOSITION AND EXUDATION FROM STORED FRUITS

TIME	EXUDATE					FRUIT		
	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE	FRESH WEIGHT	DRY WEIGHT	DRY PERCENTAGE OF FRESH
min.	*gm.*	*gm.*	*per cent.*	*sq. mm.*	*cm. per min.*	*gm.*	*gm.*	*per cent.*
0–2	0.2037	0.0193	9.4	1.77	5.7	173.0	7.00	4.05
						225.5	9.12	4.04

less elastic turgid phloem; as soon as this became emptied, flow rapidly decreased.

It seemed desirable to know the osmotic concentration of this phloem exudate, since the pressures developed depend so largely upon it. As only small amounts of the material were available, the vapor pressure method suggested by BARGER (**1**) and used on plant sap by HALKET (**17**) was used. The first determinations were made at Columbus, Ohio, on *Echinocystis lobata*. All determinations were run in duplicate. The results are shown in table XII.

TABLE XII

OSMOTIC PRESSURE DETERMINATIONS ON PHLOEM EXUDATE OF *Echinocystis lobata*

	JULY 3, 2:00 P.M.	JULY 7, 8:30 A.M.	JULY 8, 1:15 P.M.	JULY 8, 4:45 P.M.
Concentration, molar.	0.33	0.30	0.40	0.40
Osmotic pressure at 25° C. atm.	8.5	7.7	10.3	10.3
Dry weight, per cent.	. . .	10.2	7.6	8.0

The concentrations recorded in table XII indicate rather high pressures. Those given were interpolated from a concentration : osmotic pressure curve constructed from the data on sucrose given in the LANDOLT-BÖRNSTEIN tables. Since sucrose solutions were used in the determinations, these should be approximately correct. Average molecular weights calculated from these values range between 200 and 400.

More extended observations have been made on exudates from cucumber and pumpkin, samples being taken from different positions on the plants (table XIII). From these figures it may be seen that there is a consis-

TABLE XIII

OSMOTIC CONCENTRATIONS AND EQUIVALENT PRESSURES AT 25° C. OF PHLOEM EXUDATES OF CUCUMBER AND PUMPKIN

PLANT	CONCENTRATION AND OSMOTIC PRESSURE AT HEIGHT AT WHICH SAMPLE WAS TAKEN				
		9′6″	6′	3′	4″
No. 1 pumpkin, 10′ in height*	Concentration, molar.	0.31	0.25	0.26	0.23
	Osmotic pressure, atm.	8.0	6.2	6.3	6.0
		7′6″	4′		4″
No. 2 pumpkin, 8′ in height*	Concentration, molar.	0.35	0.32		0.21
	Osmotic pressure, atm.	9.0	8.2		5.4
		3′6″	2′		4″
No. 3 cucumber, 4′ in height*	Concentration, molar.	0.31	0.29		0.23
	Osmotic pressure, atm.	8.0	7.4		6.0
		5′6″	4′6″	3′	4″
No. 4 cucumber, 6′ in height*	Concentration, molar.	0.29	0.28	0.27	0.24
	Osmotic pressure, atm.	7.5	7.2	7.0	6.2
		5′6″	3′		4″
No. 5 cucumber, 6′ in height*	Concentration, molar.	0.30	0.30		0.22
	Osmotic pressure, atm.	7.7	7.7		5.7

* Plants growing in pots in the greenhouse and trained vertically on strings.

tent gradient in osmotic concentration in the exudate taken from these plants, the highest concentration coming from the region behind the zone of elongation. This shows that, at least for these plants, a concentration gradient actually exists in the conducting tracts, a relation which has been inferred from analytical data in many cases (**26, 27, 5**). The samples were very small, so that this can hardly be a case of dilution such as occurs when prolonged exudation is induced by repeated cutting of the stem.

Discussion

The possibility of a relationship between phloem exudation and translocation is obvious. The analyses of ZACHARIAS (**42**) and KRAUS (**23**) indicate that the exudate from cucurbits contains sugars, organic nitrogen compounds, potash, and phosphorus. In their late publication on translocation, MASON and MASKELL (**28**) show that these are the four types of material which accumulate above a ring in the cotton plant, and that they correspond roughly in relative amounts to those found in the above mentioned analyses.

Experiments with prolonged exudation prove that these materials move over long distances in a relatively short time. Osmotic pressure determinations indicate the sources of pressure, and dissection studies denote that the pressure is acting through the total phloem since exudation is as rapid from a tangential cut as from a transverse one. The evidence points toward the type of mechanism previously proposed (5), and the most serious remaining problem is that of locating the channels of transport.

Previous workers have agreed fairly well upon the sieve tubes, yet it remains to be proved that they serve as conducting vessels. Many of the structures which have been cited as evidence relating to flow through these cells, that is, slime accumulations, slime bubbles or droplets, and definitive callus, can hardly justify the significance given them. The median nodules and protoplasm in the pores of the sieve plates, on the other hand, should render these structures relatively impervious to rapid mass flow; yet MÜNCH mentions exudation from the sugar pine, where they undoubtedly exist. The variety of conditions under which exudation occurs, from young growing tips lacking differentiated sieve tubes to stored fruits where the sieve plates are covered with definitive callus or the tubes filled with a dense granular content, leaves but one obvious possibility. The only continuous phase occurring under all of these conditions and occupying a reasonable volume is the cell wall. It is highly hydrated, readily permeable, and continuous throughout the plant.

Studies with polarized light show the phloem walls to be doubly refractive, indicating a certain regularity of structure; and measurements of the relative lateral and longitudinal shrinkage with dehydration (5) suggest the models of SPONSLER and DORE (39). Resistance to diffusion by the walls is slight compared with that of active protoplasm, as shown by many plasmolysis tests, but the resistance to a rapid flow of solution remains to be measured.

In certain border-line cases the distinctions between diffusional movement and mass flow break down, and in this limiting condition flow seems to consist of a movement of nutrients along a diffusion gradient accompanied by movement of water along a pressure gradient. The gradient of water may be immensely steepened by increasing the pressure. From the experiments on continued exudation, it seems possible that this accelerated flow of water may carry solutes with it at a higher rate than that of natural diffusion, and so function effectively in translocation.

The rates and pressures presented in the previous section may be used to test the various theories on translocation as to their ability to satisfy requirements. Previous work (5) has indicated rates of linear displacement of the total phloem volume of about 0.3 cm. per minute in the stolon of the developing potato tuber and peduncle of the pumpkin. In table VII of the previous section, data are given which permit the calculation of rates, and these turned out to be approximately 0.4 cm. per minute during maximum growth and an average rate of nearly 0.3 cm. per minute throughout the growth period. The rates of flow measured in the exudation studies are much greater than this. Dividing the total fresh weight of exudate in table VIII by the total phloem area, an average rate of 5.0 cm. per minute is obtained.

If this rate is used in the POISEUILLE formula and the proper values inserted for the other factors, the pressure necessary to cause this flow through the sieve tubes may be determined:

$$R_1 = \frac{pr^2}{8l\eta}$$

where

R_1 = linear velocity of flow in cm. per sec.
p = pressure in dynes per $cm.^2$
r = radius of the capillary.
l = length of the capillary.
η = viscosity of the flowing solution.

Using previously determined values (5), the sieve tubes occupy about 20 per cent. of the phloem and the pores about 8.0 per cent. of the sieve plates, or 1.6 per cent. of the total phloem. Then R_1 would be 5.2 cm. per sec. through the pores of the sieve plate. The radius of the pores is 1µ or less and the length would be about 2 per cent. of the mean length of 50 cm., or 1 cm. The viscosity would not be less than that for a 10 per cent. sucrose solution, or 0. 015.

Then

$$p = \frac{8 \times 1 \times 0.015 \times 5.2}{1 \times 10^{-8}} = 62.5 \times 10^6 \text{ dynes,}$$

or 61.8 atmospheres. Since an average of the values given in table VIII indicates that only seven atmospheres are available, the exudate collected in these experiments could not have flowed through the perforations in the sieve plates even if they were free of the protoplasmic strands which traverse them.

Considering next the protoplasmic streaming hypothesis, we can see from figure 29 that the protoplasmic lining of the cell walls is very thin. If the walls occupy 30 per cent. of the phloem area (5), then the protoplasm must occupy about 20 per cent. of this, or 6 per cent.; and since only 50 per cent. of this is streaming in the proper direction at any one time, then 3 per cent. of the total phloem content is available to accelerate movement. Since R_1 for normal sap flow is 0.3 cm. per minute or 18 cm. per hour, it would be roughly 10 per cent. of this or 1.8 cm. per hour for the dry matter being moved. Dividing 1.8 by 0.03, we find that pure solute would have to move at a rate of 60 cm. per hour or at three times the maximum observed rate of streaming through the total space occupied by protoplasm in the phloem. Assuming that protoplasm contains about 80 per cent. water, it can readily be seen that protoplasmic streaming is unsatisfactory, for the total volume occupied by the solids of the protoplasm would need to be occupied by pure assimilate moving at fifteen times the maximum rate of streaming. Diffusion through end walls from cell to cell would be an additional difficulty, since the gradient across any one of the multitude of membranes would be almost negligible. MASON and MASKELL (**28**) find a similar disparity between streaming and conduction.

Testing next the possibility for flow through the walls, substitution in the formula gives the minimum size of capillary through which the normal rate of flow could take place.

$R_1 = 0.3 \div 0.15 \div 60 = 0.033$ cm. per sec. through the area of the phloem walls occupied by water.

$p = 7.0$ atmospheres.

$l = 50$ cm.

$\eta = 0.015$.

$$r^2 = \frac{8 \times 0.033 \times 0.015 \times 50}{7.0 \times 980 \times 1033} = 2.8 \times 10^{-8} \text{ cm.}^2$$

$r = 1.67 \times 10^{-4}$ cm. and the diameter of the capillary would be 3.3μ.

If flow were taking place between concentric cylinders, or in the limiting case, parallel planes, the formula becomes

$$d^2 = \frac{3\, R_1\, l \eta}{p}$$

where $2\,d =$ the distance between the planes. Substituting as before

$$d^2 = \frac{3 \times 0.033 \times 0.015 \times 50}{7.0 \times 980 \times 1033} = 1.04 \times 10^{-8} \text{ cm}^2.$$

Then

$$d = 1.02 \times 10^{-4} \text{ cm., } 2\,d = 2.04 \times 10^{-4} \text{ cm., or } 2.04\ \mu.$$

Since both the diameter for the minimal capillary and the minimal distance between parallel planes are much larger than would be expected for pore spaces in a jell, it becomes evident either that flow through the walls does not obey the viscosity formula or that some other mechanism is involved.

The studies made on the penetration and accumulation of stains by sieve tubes suggest the possibility of another explanation for translocation through the phloem. In young active sieve tubes exhibiting protoplasmic streaming, neutral red is readily accumulated but anilin blue penetrates with great difficulty. As the protoplast matures, neutral red is accumulated less and less and anilin blue penetrates more and more readily, staining slime drops, nucleus, and the callus of the sieve plate. Finally, after the slime drops and nucleus disappear, neutral red is no longer accumulated, the cells can no longer be plasmolyzed with sucrose solutions, and anilin blue penetrates readily.

Although not providing proof, these observations indicate an increasing permeability of the protoplasm of the sieve tube with maturity. If this is the case, there is the possibility that flow actually occurs partly in the walls and partly in the lumina of the sieve tubes, at least in those portions of the plant where mature sieve tubes exist. If this were the case, all that would be necessary in order that the sieve tubes show their usual characteristics is that they maintain a slight excess of turgor pressure above the hydrostatic pressure present throughout the phloem. This they apparently do, for in fresh sections of living material, although failing to plasmolyze in hypertonic sucrose solution, they appear to maintain turgor unless injured in some way in the preparation of the section.

Granting that such a combination occurs and that flow takes place partially through sieve-tube segments, it still seems improbable that the sieve plate is adapted to facilitate movement. Entry and exit would more likely take place between the protoplasmic connections and along the side walls. Certain microchemical tests indicate the presence of soluble reducing substances in phloem walls which disappear upon leaching. If leakage from sieve tubes actually occurs, an explanation of their presence is greatly simplified.

The mechanism of MÜNCH, namely, movement from living cell to living cell by way of the plasmodesma, seems adequate in the mesophyll of the leaf because of the short distances and enormous surfaces exposed. If then the solution of assimilate passes from palisade to spongy parenchyma and thence by the border parenchyma to the phloem, and there is released into cells with increasing permeability, it should slowly leak from these cells into the walls. By virtue of its osmotic activity it should continue to increase in volume until equilibrium is reached, and should flow in whichever direction the pressure gradient lies. The only other restriction would be the external limiting layer of the phloem. Thus it could flow to the growing point to supply nutrients to the developing stem, or to the root where it would nourish the meristems and supply storage organs.

Transport into the wood and out to the cortical tissues of the root appears again to be a case of movement from living cell to living cell, and wherever this type of movement occurs protoplasmic streaming should aid materially in accelerating transfer. In longitudinal movement through the petiole and stem, however, it would seem totally inadequate, as it also would in explaining the exudation from cut stems observed in these studies.

As the mechanism pictured here is developed, it can be seen to differ radically from that of **Münch**. The latter works between the turgor pressure of the assimilating cell and that of the utilizing cell. If this latter happened to be a meristematic cell of the growing point or cambium, it might have a higher turgor than the assimilating cell and translocation would become difficult to explain. In the mechanism herein pictured, the pressure gradient runs from the turgor of the assimilating cell to zero in the limiting case, and to a pressure approaching zero in all cases, it being assumed that the active growing or storing cell can maintain a low concentration of nutrients in its inclosing walls, leaving the water free to move where it will. This type of mechanism seems to fit more of the data and satisfy more of the requirements than many of the previously described theories. **Mason** and **Maskell** (**28**) object to the mechanism of **Münch**, feeling that the various substances which they have studied move independently, depending upon individual concentration gradients. When one considers the complexity of the system through which materials are moving in the mechanism being considered, it seems possible that this independence of movement may be only apparent. As the mixture of nutrients leaves the assimilating cells of the leaf, it is subject to losses all along the way from absorption by living cells. There is the possibility of increase in inorganic ions by diffusion across the cambium, as well as loss to the wood of all materials by leakage along the rays. Calcium is combined with organic acids in various regions and rendered immobile; sugar is respired at different rates in different tissues; meristematic activity occurs along the total line of transport while storage may be distinctly localized. Under this variety of conditions it would be difficult to determine the relation of any one constituent to the rate of flow or composition of the total mixture.

With the possibility of movement through both sieve-tube lumina and phloem walls, the relative importance of these depends upon the resistance which each presents to mass flow. These resistances remain to be determined by future work.

Summary

1. The theory that translocation of organic foods in plants occurs in sieve tubes and is facilitated by perforation of the end walls was developed by **Hartig**, **Nägeli**, **von Mohl**, and other early botanists and persists as the prevailing concept today.

2. Anatomists have considered the sieve plate to be perforated as an adaptation to rapid flow and few physiologists have questioned this mechanism.

3. Protoplasmic streaming has been suggested as an accelerating mechanism in translocation in sieve tubes.

4. KUHLA, SCHMIDT, RUHLAND, BIRCH-HIRSCHFELD, DIXON, BALL, and CRAFTS have raised various points in contradiction to this classical theory.

5. Sieve tubes in cucurbits are of two types: (*a*) central tubes of the phloem and (*b*) peripheral tubes of the phloem, commissural, entocyclic, and ectocyclic tubes.

6. Type *a* tubes have short, broad elements, an internal protoplasmic mesh in mature stages, and a low coagulable content. Slime plugs and definitive callus occur under certain conditions.

7. Type *b* tubes vary greatly in length but tend to be uniform in diameter. Internal protoplasmic strands are limited to sieve plates, and in mature stages these elements have a dense granular content. Slime plugs have not been found, and definitive callus appears only after obliteration or partial collapse.

8. Slime drops occur in all young sieve tubes. The coagulable contents of type *a* tubes and the granular material in type *b* tubes seem related to distintegration of these structures.

9. Protoplasmic connections traverse the sieve plates of mature sieve tubes, connecting the successive elements. While each strand is surrounded by a callus cylinder, no central pore has been found.

10. Definitive callus is formed by elongation and lateral fusion of the callus cylinders, the protoplasmic connections being stretched in the process. No callus "plugs" capable of "stopping" flow have been found.

11. Slime plugs are caused by killing reagents or gravitation and are not related to normal sap flow.

12. Mucous droplets or bubbles occur only near cuts and they would seem to hinder mass flow.

13. Protoplasmic connections of the sieve regions are intimately related to the parietal cytoplasm and appear to have a protoplasmic content.

14. Exudation from cut stems is practically limited to the phloem, except at the growing tips.

15. Exudation from the cut peduncle of a fruit stored for six weeks was normal although the sieve tubes were heavily callused or had dense granular contents.

16. Local chilling of cucumber stems resulted in a 55.7 per cent. decrease in exudation rate. Cooling the entire plant to 17° C. reduced this rate even more. Local chilling did not affect dry weight of the exudate or exudation rate from unchilled portions.

17. Calculations indicate a sap flow in cucumber equivalent to displacement of the total phloem at a rate of 0.24 cm. per minute. Developing fruits have a dry weight of about 3.9 per cent. Exudate from these fruits has an average dry weight of 7.7 per cent. Exudation from cut peduncles is very rapid.

18. Exudates from pumpkin and cucumber have an osmotic concentration equivalent to an average pressure of 7.0 atmospheres. Concentration decreases from the tips toward the roots.

19. The only continuous permeable phase throughout the plant is the cell wall. In phloem it is highly hydrated, shows regularity of structure, and occupies about 30 per cent. of the total volume in fresh sections.

20. Calculations indicate that exudation from cut peduncles cannot be explained on the basis of mass flow through perforations in the sieve plates.

21. In view of the increasing permeability of the sieve-tube cytoplasm, as indicated by staining and plasmolysis tests, it seems possible that movement may take place partly through sieve-tube lumina and partly through phloem walls.

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LABORATORY OF PLANT PHYSIOLOGY,
CORNELL UNIVERSITY,
ITHACA, N. Y.

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EXPLANATION OF PLATES IV–IX

PLATE IV

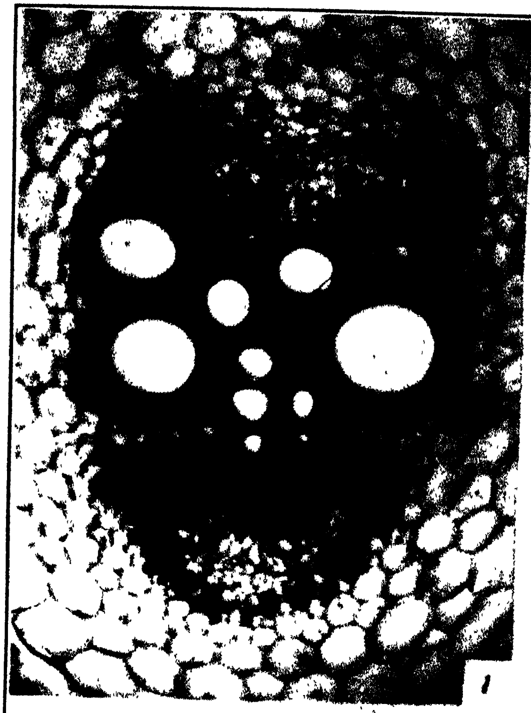
FIG. 1. Transverse section of vascular bundle of pumpkin stem killed in hot water and stained with water blue. × 75.

FIG. 2. Longitudinal tangential section of pumpkin stem killed in hot water and stained with water blue. Peripheral tubes of phloem on the left; commissural tube traversing parenchyma tissue, and entocyclic sieve tubes on the right. × 75.

FIG. 3. Longitudinal section of cucumber stem showing slime drops fixed in 50 per cent. alcohol mordanted with IKI solution and stained with water blue. Slime accumulation in mature sieve tube on the right. × 330.

FIGS. 4, 5. Transverse sections of cucumber (4) and pumpkin (5) stems showing slime drops in parietal protoplasmic layer. Killed in hot water fixed with alcohol and stained with water blue. × 690.

PLATE IV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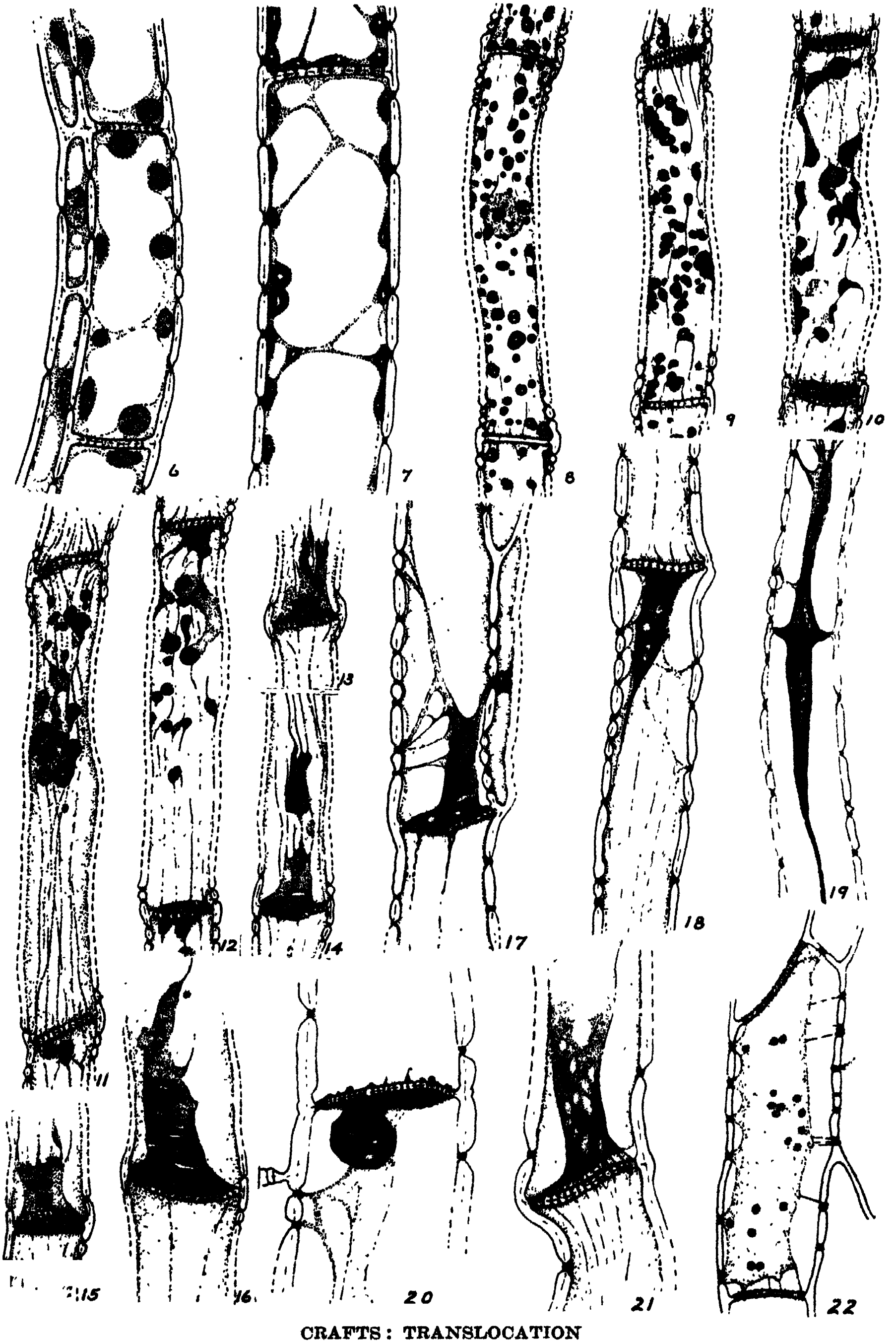

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PLATE V

FIG. 6. Freehand drawing showing optical section through young living sieve tube and companion cell of cucumber stained with neutral red. The protoplasm was streaming and constantly changing in form. × 400.

FIG. 7. Similar illustration of more mature sieve tube. The protoplasm was showing plastic movements and strands were narrow, often stretching and breaking while the section was under observation. × 400.

FIGS. 8–12. Camera lucida drawings of sieve tubes of cucumber showing slime drops in successive stages of development. Fig. 8, young stage; fig. 12, slime drops disintegrating. The internal protoplasmic strands are threadlike in fixed material. From stems killed in hot water, fixed in 50 per cent. alcohol and stained with water blue. × 175.

FIGS. 13–15. Slime drops accumulated in sieve tubes of cucumber stems cut and placed in boiling water. The sieve plates illustrated were near the cut end and accumulation was due to the killing action of the hot water. Sections fixed in alcohol and stained with water blue. × 175.

FIG. 16. Slime accumulation in mature sieve tube of cucumber after disintegration of slime drops. Section from stem treated as in previous figures. × 175.

FIGS. 17, 18. Slime accumulation in sieve tubes of cucumber stems killed and fixed in 50 per cent. alcohol. Parts of the internal protoplasmic structure adhering to the pits in side walls. × 400.

FIGS. 19, 20. Slime accumulation in sieve tubes of cucumber stems killed and fixed in 95 per cent. alcohol. In figure 19 the parietal protoplasm has contracted and closely surrounds the coagulated slime; in figure 20 the violent contraction of the protoplasmic structures has entirely ruptured them on upper side of plate, while on lower side remnants of parietal layer remain; inner threads have contracted and are hidden within the mass of coagulated slime. Fig. 19, × 175; fig. 20, × 400.

FIG. 21. Sieve tube of pumpkin stem killed in hot water, showing protoplasmic connections drawn out by slight contraction of protoplasm. × 400.

FIG. 22. Cell of epidermis of vein from dorsal side of cucumber leaf stained with pyoktanin blue to show protoplasmic connections. Protoplast was plasmolyzed and threads remain connected to pits. × 175.

PLATE VI

FIG. 23. Sieve plate in cucumber showing protoplasmic connections as black spots surrounded by callus cylinders. × 690.

FIG. 24. Same sieve plate with focus raised to show the protoplasmic strands as light spots surrounded by the darker callus cylinders. × 690.

FIG. 25. Same sieve plate with side illumination to bring out the crater-like appearance of ends of the callus cylinders. × 690.

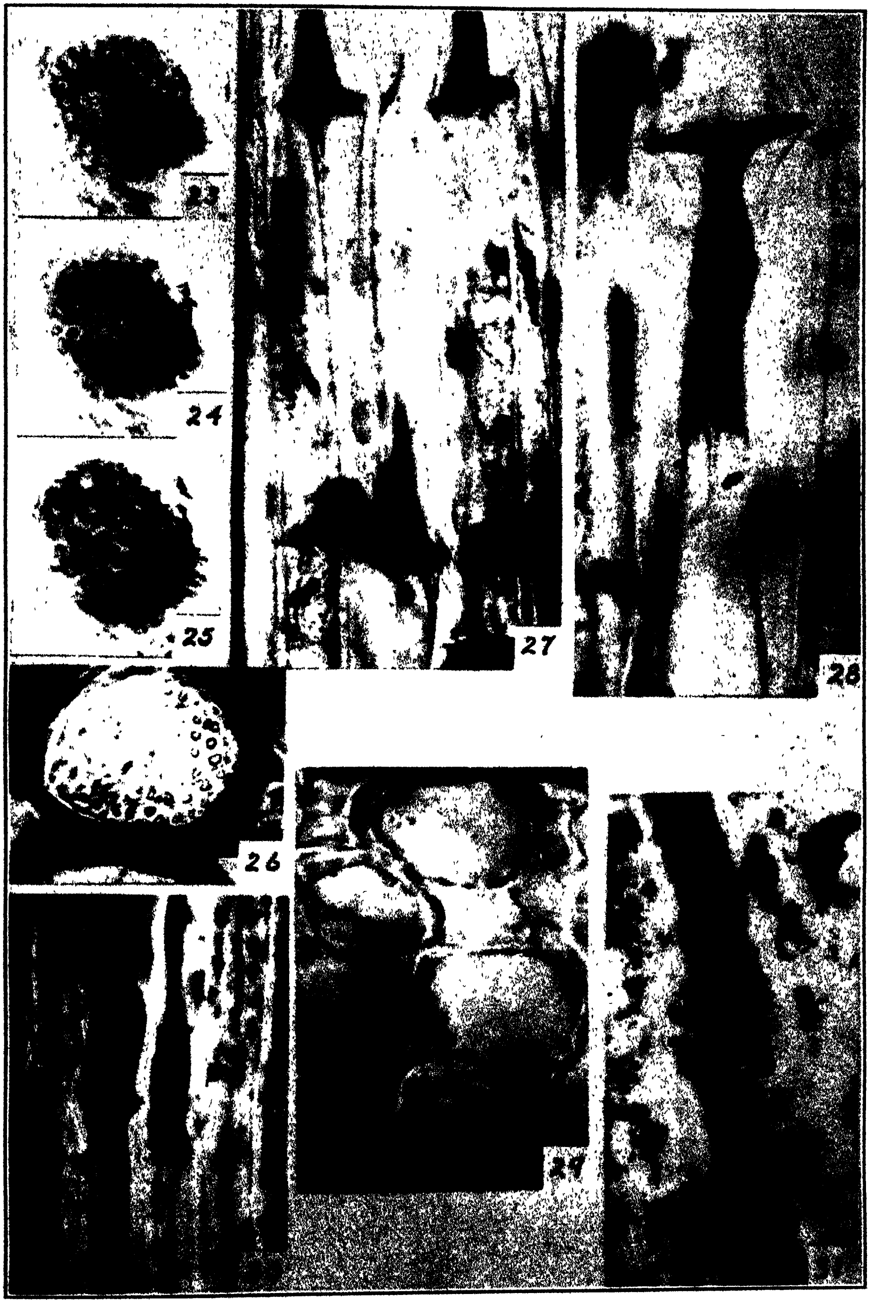
FIG. 26. Young sieve plate in pumpkin stem showing initiation of the callus cylinders surrounding protoplasmic connections. × 400.

FIGS. 27, 28. Mature sieve tubes of cucumber stem killed in 50 per cent. alcohol, showing slime accumulations, protoplasmic strings, and parietal protoplasm. Fig. 27, × 330; fig. 28, × 690.

FIG. 29. Transverse section of pumpkin stem showing thin parietal protoplasmic layer slightly plasmolyzed in places. × 540.

FIG. 30. Slime accumulations in section of pumpkin stem. The section on the slide was touched on each end with a hot needle and this figure shows the resulting slime accumulations on both sides of same sieve plate. × 690.

FIG. 31. Callused sieve plate from old pumpkin stem stained with pyoktanin blue to show protoplasmic connections. × 690.



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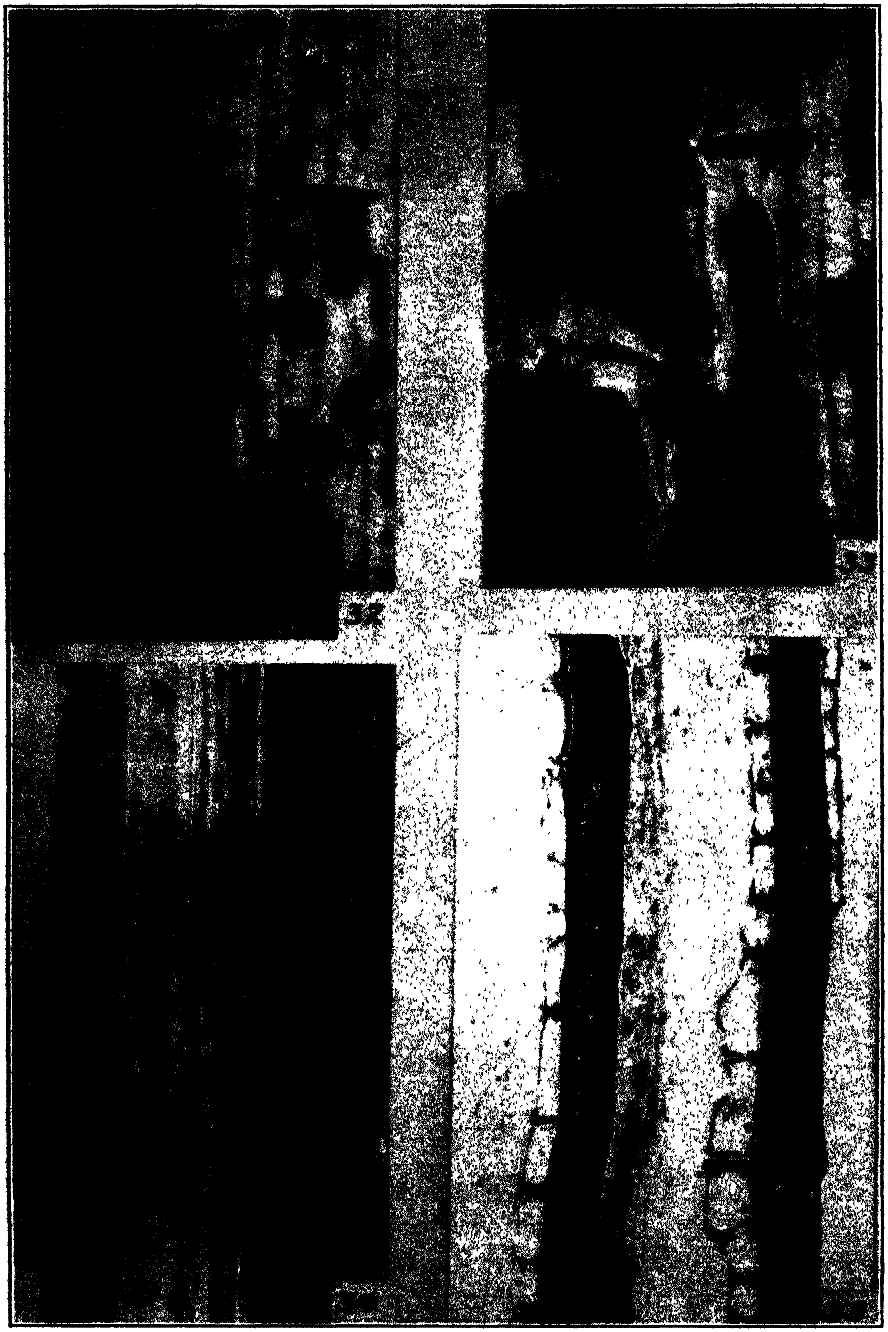

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PLATE VII

FIG. 32. Slime accumulation in cucumber stem killed and fixed in 50 per cent. alcohol. Pieces of stem about 6 mm. in length were placed in the alcohol for 30 minutes and then sectioned. In the portion midway between the two ends, slime accumulations occurred in various positions in the sieve-tube elements. At cut ends they were present on the sieve plates on the side away from the cut. × 330.

FIG. 33. Slime accumulation in section of cucumber stem treated on the slide with a hot needle, illustrating condition at a distance from the treated end. × 690.

FIG. 34. Entocyclic sieve tube of pumpkin in slime drop stage. Slight plasmolysis in the lower element brings out relation of the slime drops to the parietal protoplasmic layer. × 330.

FIG. 35. Sieve tubes at periphery of the phloem group (*cf.* with figs. 1 and 2) of same stem as shown in figure 34. These tubes are more mature but do not yet have definitive callus on their sieve plates. × 330.

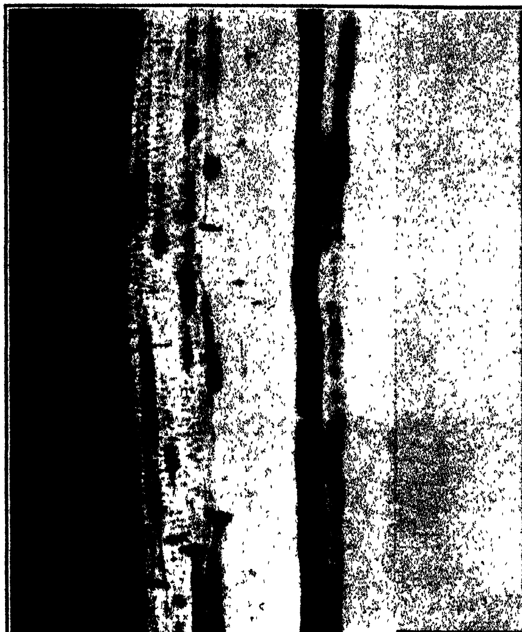
PLATE VIII

FIG. 36. Pumpkin stem killed in hot water, showing densely filled peripheral sieve tube on right and central tubes with slime accumulations in center. × 150.

FIG. 37. Entocyclic sieve tube of cucumber with definitive callus on sieve plate and having cracks in the dense amorphous contents. × 410.

FIG. 38. Entocyclic sieve tube of pumpkin with amorphous mass at the sieve plate and granular content in remainder of tube. × 330.

FIG. 39. Peripheral tubes in same stem as illustrated in figure 38. Amorphous material lines the walls, leaving granular core in center of the tubes. Contents of tube on right have become completely amorphous but have not yet cracked as in figure 37. × 330.



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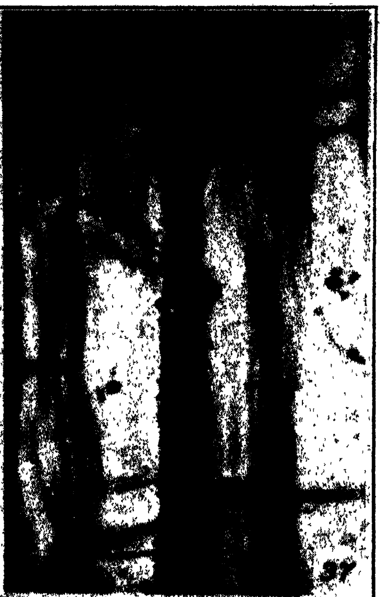

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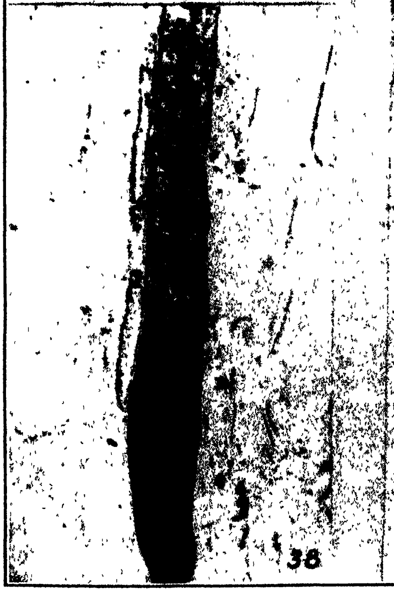

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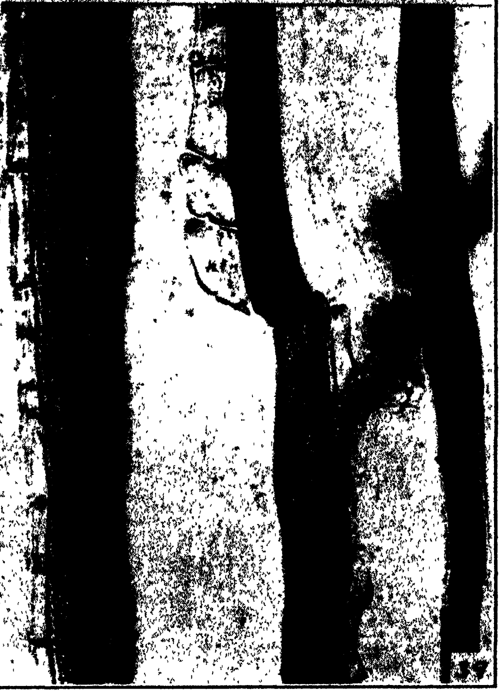

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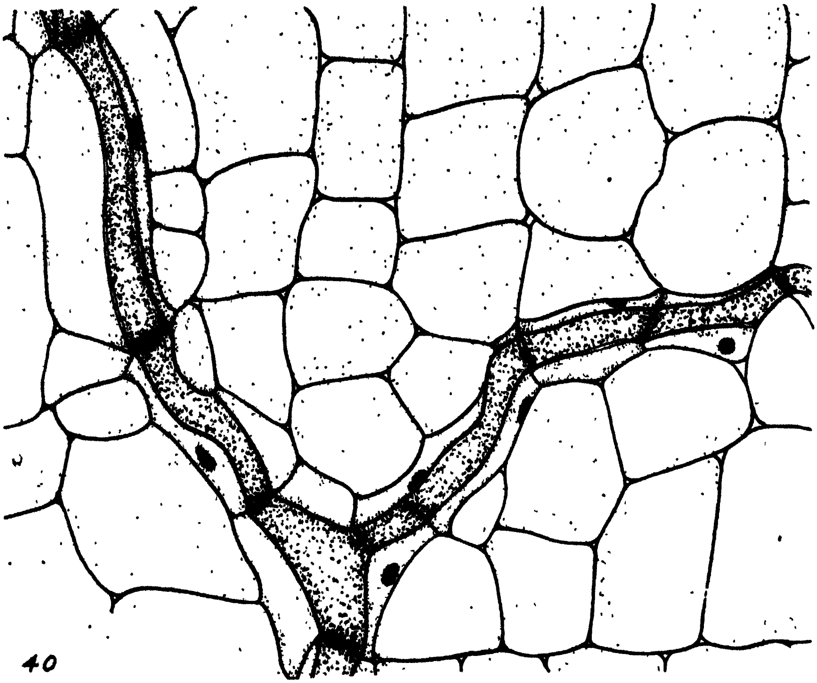

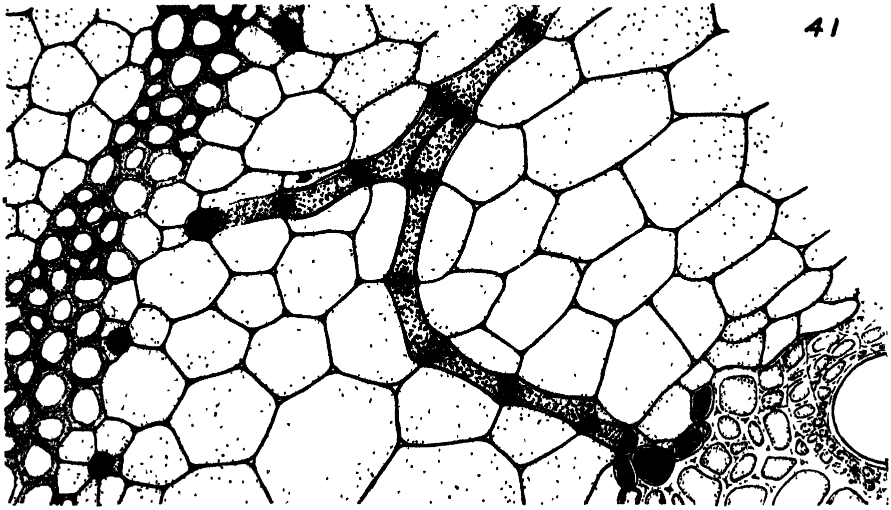

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PLATE IX

FIG. 40. Camera lucida drawing of longitudinal section of cucumber stem showing commissural sieve tubes traversing parenchyma tissue. × 96.

FIG. 41. Camera lucida drawing of transverse section of cucumber stem showing relation of commissural tubes to phloem groups and entocyclic tubes. × 96.

FACTORS AFFECTING THE DISTRIBUTION OF IRON IN PLANTS[1]

C. H. ROGERS AND J. W. SHIVE

(WITH EIGHT FIGURES)

I. Introduction

The influence of acid and alkaline reactions of the growth medium on iron relationships in plants has been known, or at least suspected, for over a century. The internal conditions of the plant in their relation to iron metabolism and chlorophyll production are not at all well understood. A number of investigations (**22, 23, 20, 18, 16, 14, 10, 3, 1**) have been carried out in which the general conclusion is that the reaction of the growth medium governs, to a large extent, the quantities of iron available to the plant. These observations are in keeping with the results obtained by PATTEN and MAINS (**24**). Working with HCl solutions containing definite concentrations of iron, they found that, beginning with a pH of 3.5, iron was precipitated in increasing amounts up to pH 6.0, above which point practically no iron remained in solution. This was accomplished by adding, in separate experiments, ammonium hydroxide, sodium hydroxide, and hydrogen sulphide to the hydrochloric acid solutions containing definite quantities of iron. The range of iron precipitation under these conditions thus extends from pH 3.5 as the lower limit to pH 6.0 as the upper limit.

All of this work deals with the influence of the reaction of the growth medium on the availability of iron. Very little has been said regarding the possible influence of internal reactions on the availability and mobility of iron within the plant. Investigations (**21, 12, 13, 17**) in which actual quantitative measurements were employed in determining iron in the plant tissues generally indicate that plants chlorotic from lack of available iron contain as much, or more, total iron per unit weight of tissue than do normal green plants of the same species. From this it follows that certain internal conditions may render unavailable to the chlorophyllous cells the iron already in the plant.

INGALLS and SHIVE (**15**) found that not only does the H-ion concentration of plant tissue fluids fluctuate over a 24-hour day and night period, but that the soluble (filterable) iron content also fluctuates, and, in most cases, is directly proportional to the H-ion concentration of the respective tissue fluids. The pH of fleshy succulents fluctuates much more than does that of thin-leaved or non-succulent forms, the H-ion concentration in all cases being higher at night and decreasing with increased light intensity. Plants with a pH of tissue fluids lying near or at the upper limits of iron precipitation show a much greater fluctuation of filterable iron content following variation in pH of tissue fluids than do plants with a pH near the lower limits of iron precipitation.

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[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

GUSTAFSON (**11**), using *Bryophyllum calycinum*, found a fluctuation of approximately two pH units over a 24-hour period. On the other hand, plants kept in darkness (all other factors being similar) showed very little or no fluctuation over the same 24-hour period.

AUBERT (**4**) was one of the pioneers who attempted to explain why plants were more acid at night than in the daytime. His work was done with cacti,—fleshy succulents in which there is considerable fluctuation in pH from day to night. He concluded that increased acidity at night is due to the formation of organic acids, chief of which is malic acid. The acid formation is the result of incomplete or partial oxidation of the more complex compounds. In the daytime, these acids are broken down by the sunlight: a photolytic process. SPOEHR (**29**), working with the same plant, arrived at conclusions similar to those of AUBERT. By the action of sunlight and a mercury arc lamp, he found that malic acid solutions are decomposed and the acidity of expressed cactus sap is greatly decreased. RICHARDS (**26**), also using the cactus, arrived at conclusions similar to those of AUBERT and SPOEHR. He thinks, however, that this partial oxidation, acid-forming process, goes on in the daytime as a part of natural processes, but is hidden, for the most part, by photolytic action, which breaks down the acids formed. WARMING (**30**) finds it generally true that in succulent xerophytic land plants the air-containing intercellular spaces are very narrow. In this connection he cites measurements by ALTENKIRCH. From these facts and the researches of AUBERT (**4**), WARMING thinks that this limited air passage does not allow for sufficient oxygen supply; hence the formation of acids due to partial oxidations.

II. Experimentation

A. INTRODUCTORY

It has frequently been found that certain plants become chlorotic, apparently from lack of iron in the chlorophyllous cells of the leaves, although excessive quantities of iron are present in the tissues of the stems and leaves of these plants. The problem here investigated is an attempt to find in just what tissues these excessive accumulations of iron occur, and to determine why such a phenomenon occurs in some plants and not in others.

Experiments were planned whereby quantitative determinations might be made for total and soluble iron content of plants having juices with very high pH values and others having tissue fluids with very low pH values, the plants being grown both in artificial media and in the open field. For determinations of soluble iron, the juices of the stems and leaves were extracted and filtered and the filtrates analyzed. Total iron was determined by analyses of the dried plant tissues. A study was then made of the relation between soluble and total iron content of these plants and the pH values of the extracted tissue fluids, to determine to what extent pH values of the extracted juices influenced distribution and availability of iron in the plants for chlorophyll production.

An investigation was also undertaken to determine the H-ion concentrations of the various tissues and of cells in sectional areas of the different organs; and to study the influence of pH values of the different tissues upon iron accumulations in them, and upon translocation and precipitation of iron in these plants. This investigation was carried out by means of microchemical methods and microscopical observation of sectional areas, which are described in the following pages.

B. TREATMENT OF SAMPLES PREPARATORY TO ANALYSIS

In order to obtain comparative results, plant samples for pH and iron determinations must be representative, and must be taken at definite intervals over a period of 24 hours, since it has been shown that the pH of plant sap varies with light intensity (**11**, **15**) and the soluble iron content varies with the pH (**15**). In the present series of experiments, it was decided to collect samples at 4-hour intervals, namely, at 10 A. M., 2 P. M., 6 P. M., 2 A. M., and 6 A. M. The collection of samples was always made on clear days, so that maximum differences in light intensity between day and night could be obtained.

The samples were broken or cut with a highly polished, stainless steel knife into small pieces and thoroughly mixed, so that representative samples for analyses could be obtained. This was the only time during the procedure that the material was allowed to come in contact with anything but glass surfaces. Part of the material collected at each interval was put into small glass dishes suitable for weighing. From this, dry weight and total iron determinations were made. The other part of each sample was put into glass vials, paraffined cork stoppers were inserted, and the whole plunged into an ice-salt mixture and frozen as soon as possible. After being thoroughly frozen, the material was allowed to thaw in warm water and the sap was extracted by means of a small handscrew press. Two or 3 cc. of the sap were poured into a short Pyrex tube, and electrometric pH determinations were made immediately. The remainder of the sap (at least 4 or 5 cc.) was filtered through a high-grade quantitative filter-paper. One cc. duplicate or triplicate samples of the filtered juices were measured into Pyrex digestion tubes immediately after filtration was completed. Iron analyses of these samples were later made. Green weights were quickly obtained and recorded, using the material set aside for this determination. These procedures were accomplished as quickly as possible after sampling, in order to avoid any appreciable physical or chemical changes before determinations were completed. After weighing the green tissue, it was dried in an electric oven at 85° C. for 48 hours, and then six to eight hours at 102°. The dry weights were recorded and this material was preserved for total iron determinations. In order to calculate the amount of filterable (soluble) iron per gram of dry tissue, the number of grams of water per gram dry weight was determined, and this figure multiplied by the figure representing the amount of iron found in the 1-cc. sample of filtered juice; the resulting figure was taken to represent the amount of soluble iron per gram of dry tissue.

The material to be collected was thoroughly washed by spraying with distilled water once the day before and again three or four hours before collecting of samples began. This was done to rid the tissue, as much as possible, of dust and dirt that might have accumulated during growth of the plants.

III. Analytical methods

A. MICROCHEMICAL

PH DETERMINATIONS.—In making pH determinations of cells and tissues, a series of indicator dyes recommended by CLARK (5) and SMALL (27) was used. The series consisted of the following eight dyes, listed with their color and pH ranges:

Meta Cresol Purple	Red 1.2–2.8 Yellow
LaMotte Yellow	Red 2.6–4.2 Yellow
Bromphenol Blue	Yellow 3.0–4.6 Blue
Bromcresol Green	Yellow 3.8–5.4 Blue
Methyl Red	Red 4.4–6.0 Yellow
Chlorphenol Red	Yellow 5.2–6.8 Red
Bromthymol Blue	Yellow 6.0–7.6 Blue
Phenol Red	Yellow 6.8–8.4 Red

Aqueous solutions of the alkali salts were prepared according to directions given by CLARK (5).

For making the actual pH determinations, free-hand sections of the tissues were made with a sectioning razor, the sections quickly rinsed in distilled water, and placed in spot plate depressions containing the dye solutions. A large spot plate with eight depressions was used, so that different sections of the same tissue could be put in all of the eight dyes at the same time. After standing half an hour or more, observations could be made. This was accomplished by quickly rinsing the sections in distilled water, placing on a slide in a drop of distilled water, and immediately observing with the microscope.

IRON TESTS.—Tests for iron were made by making free-hand sections with a high quality, highly polished, stainless steel sectioning razor, quickly rinsing in distilled water, treating with a dilute (1–15) solution of hydrochloric acid, and then from 15 to 30 minutes with a 1.5 per cent. solution of potassium ferrocyanide. In the presence of iron, a blue precipitate is formed. This iron complex, commonly called Prussian blue or Berlin blue, is ferric ferrocyanide, and is formed in the presence of ferric iron. Ferrous iron may be determined by using potassium ferricyanide, but for total iron (ferrous and ferric iron) this is unnecessary, since the ferrous ferrocyanide formed in the presence of ferrocyanide and ferrous iron soon oxidizes to the ferric ferrocyanide. These tests may also be made either directly on the micro slide, or in watch glasses and then transferred to the slide. In all of the microchemical tests, the sections were handled with glass-tipped forceps.

B. MACROCHEMICAL

pH DETERMINATIONS.—pH determinations were made on about 3 cc. of extracted sap, by means of a hydrogen electrode and Type K Leeds and Northrup potentiometer. Hydrogen was bubbled through the solution until a constant potential was obtained, a procedure which usually required about 40 seconds. The electrode was cleaned and blacked frequently and checked against a standard buffer solution at frequent intervals throughout the work.

IRON ANALYSES.—Iron analyses were made according to the WONG (**31**) method, somewhat modified, which consisted of thoroughly digesting the sample with sulphuric acid, oxidizing with sodium chlorate, and adding potassium thiocyanate; after which the contents were made to a known volume and colorimetrically compared with a known standard solution. The red coloration obtained is due to the formation of ferric thiocyanate. The analyses were made in Pyrex digestion tubes, graduated to 12.5 cc. and 25 cc. Details of the method are given by INGALLS and SHIVE (**15**), and may therefore be omitted here.

The material for total iron was furnished by that used in making moisture and dry weight determinations. This material was ground to a powder with a mortar and pestle and allowed to dry for a day, first in the oven at 75° C. for six hours, and then in a desiccator. One-tenth gram samples were accurately weighed and transferred to the previously mentioned graduated Pyrex digestion tubes.

IV. Influence of H-ion concentration on soluble and total iron content

The purpose of these experiments was to study the relations of H-ion concentration to total iron and soluble iron in plants with juices having very high and very low pH reactions, and to determine whether the filterable (soluble) iron content of such plants remains fairly constant over a 24-hour day and night period under these conditions of very high and very low pH reactions. In accordance with the work of PATTEN and MAINS (**24**), in which they found that all iron was precipitated from a hydrochloric acid solution at and above a pH of 6.0, while all iron remained in solution below a pH of 3.5, it might be inferred that plant juices with a pH above 6.0 contain no soluble iron, and those with a pH below 3.5 contain iron in the soluble form only. From the data presented in these experiments, it becomes apparent that the results obtained by PATTEN and MAINS with hydrochloric acid solutions cannot be applied to extracted plant juices.

By determining the pH values of the expressed juices of a number of species, it was found that the juices of *Oxalis repens* and *Rumex acetosella* had the lowest pH values of any species tested, while *Solanum tuberosum* and *Glycine max* juices had rather high pH values. These plants were grown in ordinary wooden flats containing a well composted and thoroughly mixed soil.

Samples of the plants were collected at 4-hour intervals over a 24-hour (day and night) period, and the juices extracted as previously described. The results of the pH and iron determinations of the juices of these plants are given in tables I and II, and the averages of these over the 24-hour period are given in table III. The data from tables I and II are illustrated graphically in figures 1 and 2 respectively.

In preparing the graphs illustrating the relations of light intensity, H-ion concentration, and soluble iron content, both iron and pH values were plotted on the same graph, using a common abscissa but different ordinates. For purposes of clearer illustration, the values for soluble iron were arranged in the inverse or descending order, since these values decrease with increasing pH values.

It has been shown by INGALLS and SHIVE (**15**), and it might well be expected, that in such a plant as *Bryophyllum*, where the pH of the plant juice fluctuates widely from near the lower limits to the upper limits of iron precipitation (pH 3.5–6.0), according to PATTEN and MAINS, the soluble iron content might also vary over a wide range during consecutive periods of high and low light intensity; and it has indeed been found that the higher the light intensity and the higher the pH of the plant tissue fluids, the lower is the soluble iron content. If the results of PATTEN and MAINS could apply to plant juices, it might then be expected that in plants which show fluctuating pH values, either entirely above the extreme upper limit (pH 6.0) of iron precipitation or entirely below the extreme lower limit (pH 3.5), there should be little or no variation in the soluble iron content with variation in light intensity and with resulting variations in pH values of tissue fluids. Again, from the experimental results presented here, it will be seen that such values do not hold true in the case of extracted plant juices.

INGALLS and SHIVE (**15**), working with nine different species with pH values ranging from 4.04 to 6.1, found the soluble iron content to vary in an inverse relation with the pH values; that is, decrease in pH values of plant juices is followed by a corresponding increase in soluble iron content, and *vice versa*. Some of the species showed much greater fluctuations in pH values and soluble iron content of the tissue fluids than did others, the fluctuation increasing with increased succulency of the plants.

OXALIS AND RUMEX

It will be observed from table I that fluctuations in the pH of the plant juices of *Oxalis* and *Rumex* do not vary through wide ranges with variation in light intensity from day to night. *Oxalis* shows a range in pH variation from 2.198 to 1.893, and the juices of *Rumex* show a variation between 2.671 and 2.468 from day to night. The filterable iron content of the *Oxalis* plant shows a corresponding range of variation, but in this plant there appears to be no relation between the variation in pH and the varia-

TABLE I

PH, FILTERABLE AND TOTAL IRON IN *Rumex* AND *Oxalis* OVER A 24-HOUR PERIOD

SPECIES	TIME OF COLLECTING	PH	IRON (MG. PER GM. DRY WEIGHT)	
			FILTERABLE	TOTAL
			mg.	*mg.*
Oxalis (leaf)	10 A. M.	1.995	0.063	0.167
	2 P. M.	1.893	0.053	0.158
	6 P. M.	1.961	0.047	0.183
	10 P. M.	2.198	0.050	0.189
	2 A. M.	2.164	0.072	0.165
	6 A. M.	1.927	0.061	0.160
Rumex (leaf)	10 A. M.	2.502	0.092	0.194
	2 P. M.	2.603	0.072	0.194
	6 P. M.	2.671	0.075	0.196
	10 P. M.	2.570	0.077	0.206
	2 A. M.	2.520	0.092	0.191
	6 A. M.	2.468	0.093	0.200

tion in soluble iron content; while *Rumex* shows the definite inverse relation between pH and filterable iron content of plant juices which has been found for many other species. It is to be emphasized that although these fluctuations are no doubt significant, they vary over a rather narrow range.

While the juices of *Rumex* at all times show pH values considerably below the lower limit (pH 3.5) of iron precipitation as indicated by PATTEN and MAINS (**24**), this plant nevertheless shows the same correlation between pH and soluble iron content of tissue fluids as do the species investigated by INGALLS and SHIVE (**15**). On the other hand, *Oxalis*, with extremely low pH values of tissue fluids, shows no such correlation, nor does there appear to be any direct relation between light intensity and pH of tissue fluids in this plant, although both pH values and soluble iron content fluctuate quite as much during a 24-hour period as do these values in *Rumex*. The relations of pH values and soluble iron content for *Rumex* are clearly indicated by the graphs of figure 1, which were plotted from the data of table I.

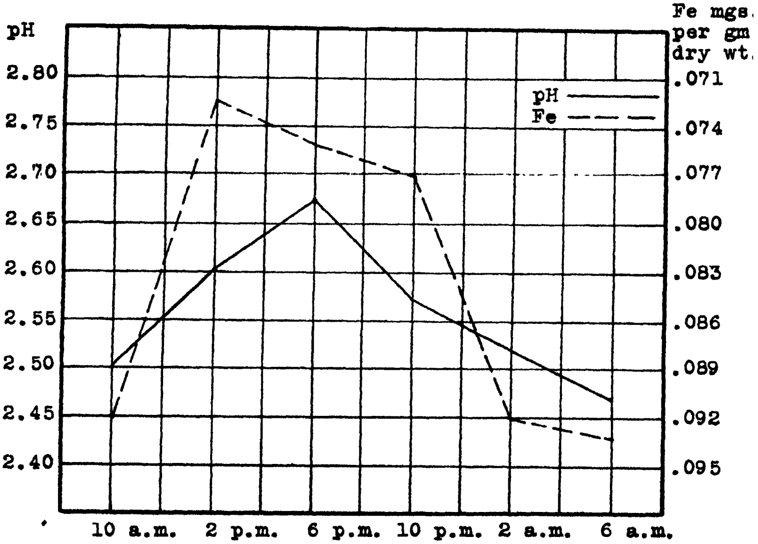

FIG. 1. Graph showing relation of pH to soluble iron content of *Rumex* leaves during 24-hour experimental period.

From the evidence obtained in the experiments with *Rumex*, it may safely be concluded that, although the pH values during a 24-hour period never reach the lowest point (pH 3.5) in the iron precipitation range, as found by PATTEN and MAINS (**24**), the soluble iron content is nevertheless directly correlated with fluctuations in the H-ion concentration.

SOY BEAN AND POTATO

As may be observed from the data presented in table II and the graphs of figure 2, which were plotted from these data, the soluble iron contents of both the potato and the soy bean plant show, during a 24-hour period, fluctuations which are closely correlated, in an inverse relation, with the fluctuations in pH values of the tissue fluids. The average pH of the potato is 6.334 and that of the soy bean is 5.996, as indicated in table III.

TABLE II

pH, FILTERABLE AND TOTAL IRON CONTENT IN POTATO AND SOY BEAN PLANTS AT DIFFERENT INTERVALS OVER A 24-HOUR PERIOD

SPECIES	TIME OF SAMPLING	pH	IRON (MG. PER GM. DRY WEIGHT) FILTERABLE	IRON (MG. PER GM. DRY WEIGHT) TOTAL
			mg.	*mg.*
Potato (leaf)	10 A. M.	6.188	0.026	0.612
	2 P. M.	6.576	0.028	0.522
	6 P. M.	6.424	0.029	0.580
	10 P. M.	6.306	0.029	0.498
	2 A. M.	6.272	0.031	0.538
	6 A. M.	6.238	0.034	0.652
Soy bean (whole plant)	10 A. M.	6.002	0.010	0.314
	2 P. M.	6.086	0.010	0.444
	6 P. M.	5.934	0.012	0.484
	10 P. M.	6.103	0.011	0.594
	2 A. M.	6.019	0.016	0.619
	6 A. M.	5.833	0.019	0.682

TABLE III

AVERAGE SOLUBLE IRON, TOTAL IRON, AND pH VALUES FOR *Oxalis*, *Rumex*, POTATO AND SOY BEAN, OVER A 24-HOUR PERIOD

SPECIES	pH	IRON SOLUBLE	IRON TOTAL
		mg.	*mg.*
Oxalis (leaf)	2.023	0.058	0.170
Rumex (leaf)	2.563	0.084	0.197
Potato (leaf)	6.334	0.029	0.567
Soy bean (whole plant)	5.996	0.013	0.522

Soluble iron content of the soy bean fluctuates very strikingly following fluctuations in the pH values of tissue fluids. In the juices of these plants, the pH varies from just above the point of complete iron precipitation (as found by PATTEN and MAINS) to just below this point. The same close relation holds for fluctuations in the potato, with the exception of the first daylight period, although no pH value is as low as the extreme upper limit of iron precipitation. From the foregoing results, it may be concluded that although the pH values of tissue fluids of certain plants may lie consider-

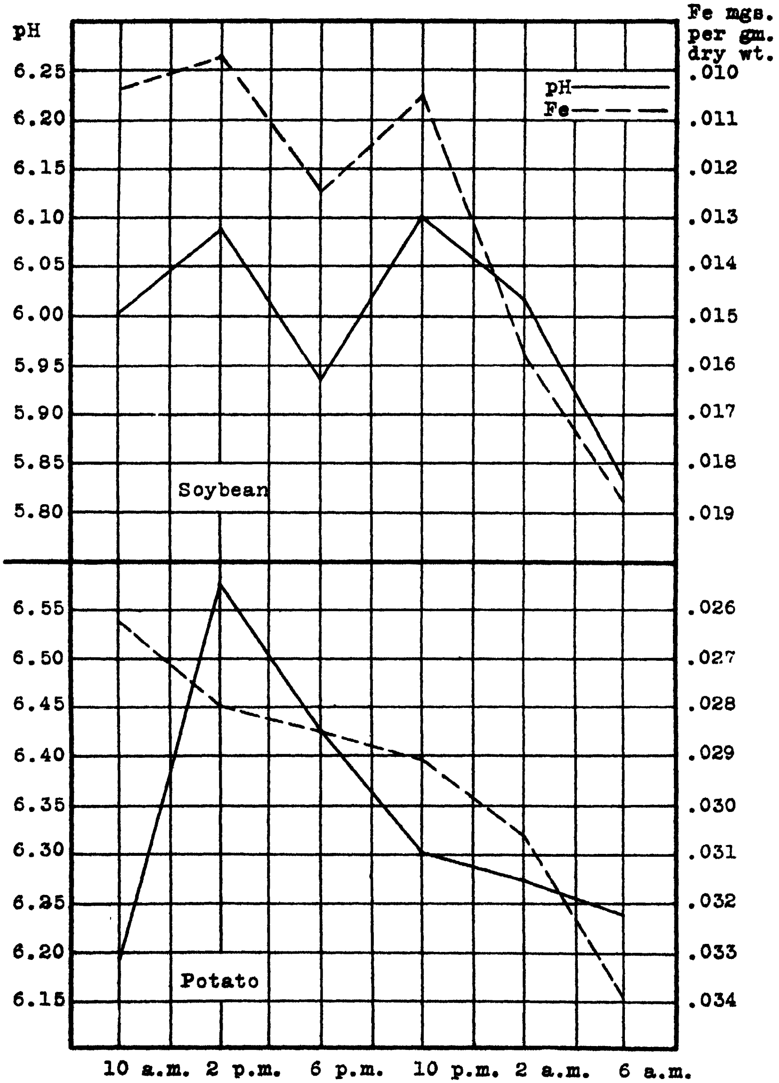

FIG. 2. Graphs showing relation of pH to soluble iron content of soy bean and of potato plants during 24-hour experimental period.

ably above the upper limit of iron precipitation in inorganic solutions, the soluble iron content still rises and falls within a narrow range with the fluctuating pH values resulting from variations in light intensity. This feature of the problem will receive more detailed consideration in a later section, but it might not be out of place here to emphasize several important points.

From the results obtained in this investigation, it is apparent that the range in pH values over which iron precipitation may proceed in simple inorganic solutions, according to the work of PATTEN and MAINS, cannot hold for complex organic systems such as are represented by the tissue fluids in plants. Plants yielding composite tissue fluids with pH values considerably above 6.0 still contain soluble iron, although in relatively low concentrations, yet in sufficient concentration to supply the needs of the plant under favorable conditions. Furthermore, plants yielding composite tissue fluids with pH values considerably below the lower limit of iron precipitation in simple inorganic solutions still contain insoluble iron, or at least iron which does not appear in the filtered plant juices with the methods here employed; although INGALLS and SHIVE (**15**) have shown that in some high-acid plants, practically all of the iron is present in the filterable form, and may be recovered in the filtrate.

It will be seen from tables I, II, and III that plants with juices having high pH values have high total iron and low soluble iron content, while plants with juices having low pH values have low total iron and high soluble iron content. This means that in plants with relatively high acid reaction, most of the iron is in solution; while in plants with relatively low acid reaction, most of the iron is in the insoluble form and accumulates as such. It is more than probable that if, for some reason, all of the iron in the soy bean or potato plant should suddenly become soluble, it would be extremely toxic to the respective plants. Even chlorotic plants, as has already been stated, may have large accumulations of iron present, even in the chlorotic leaves; but this iron may still be unavailable for the processes requiring it in the chlorophyllous cells. Two important questions arise: Where and why do such accumulations of iron occur? Consideration of these questions will be attempted in the following section.

V. H-ion concentration and distribution of iron as determined by microchemical examination of sectional areas

In carrying out this investigation, it was assumed that a microchemical study of sectional areas of plant tissues might bring out some facts with regard to H-ion concentration of tissues, and its relation to distribution of iron, which could not be obtained by making chemical analyses of tissues of different plant organs and determining the pH values of their juices. This proved to be the case.

In these studies, fresh tissue sections were prepared, microchemical tests were made for iron, and pH values of the different tissues over these sectional areas were determined as previously described. Plants were purposely chosen which yielded composite juices having very low and very high pH values. Among the former were *Oxalis repens* and *Rumex acetosella,* and among the latter were such plants as *Zea mays, Solanum tuberosum, Trifolium repens,* and *Glycine max.*

Sections were prepared from plants grown in the field, and also from those grown under controlled experimental conditions in the greenhouse. Great numbers of these tissue sections were prepared and studied, but only a few photographs (made from camera lucida drawings) of representative sectional areas are here presented and discussed. In the diagrams of these sectional areas, iron, as determined by the microchemical tests employed, is indicated in the cells of the different tissues by stippling, and the attempt is thus made to show approximately the relative quantities of iron present in the cells of the various tissues as these were made apparent by microscopical examinations of the sections after chemical treatment. The methods are strictly qualitative, however, and must be so regarded; they do not differentiate between iron in the soluble form and as a precipitate.

The methods employed in the determination of pH values of the different tissues and cells in sectional areas may not be regarded as being particularly accurate, nor has any method yet been devised by means of which great accuracy may be attained in determining pH values of tissues or cells in cross-section. An attempt has been made, however, to determine two values between which it may be regarded that the true pH of the tissues or cells in question is located. In the following consideration of data relating to sectional areas of plant tissues, two such values are always presented.

OXALIS.—The data representing the pH values of the different tissues in a cross-section of the stem of *Oxalis* are presented in table IV. These data indicate that the pH of all the tissues is relatively very low, the values

TABLE IV

APPROXIMATE PH VALUES OF VARIOUS TISSUES OF THE STEM OF *Oxalis* STUDIED IN CROSS-SECTION

TISSUES	PH VALUES	TISSUES	PH VALUES
Cortex	4.2–3.8	Cambium	.
Epidermis	2.8–2.6	Xylem	3.0–2.4
Endodermis	2.8–2.2	Pith	4.2–3.8
Phloem	4.4–3.8		

lying between pH 2.8 and 2.2 for endodermis cells, and between pH 4.4 and 3.8 for phloem. The average pH of the composite tissue fluid extracted from the stem of this plant, as previously given, is pH 2.023.

Examination of the diagram of figure 3, showing a cross-section of the stem of *Oxalis,* brings out the fact that the iron in this stem, as determined by microchemical methods, is rather uniformly scattered in all the tissues, and that the iron content of all the tissues is apparently very low, as this is determined by the methods here employed. This uniform distribution and low iron content are characteristic of the conditions throughout the plant. Accumulations of iron were not found anywhere in the tissues of this plant, except for slight accumulations which were sometimes observed in rather large isodiametric cells in the outer cortex.

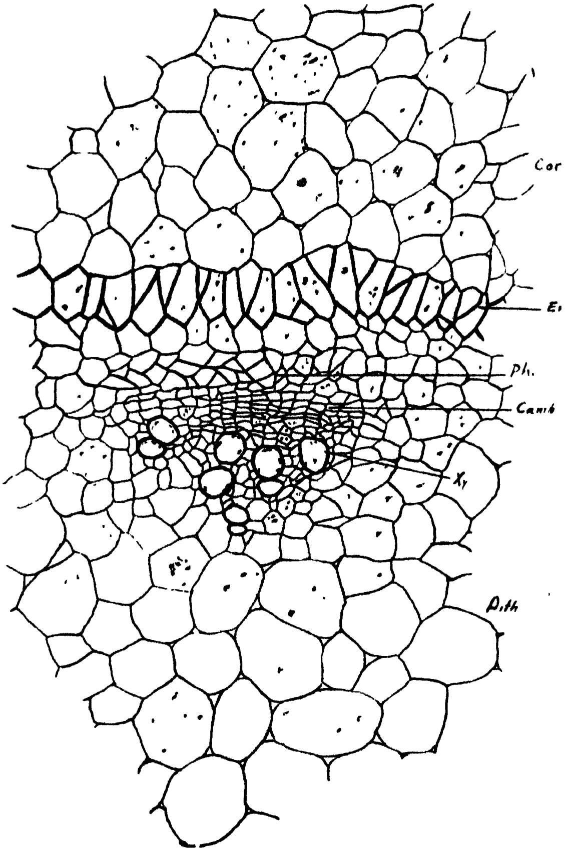

FIG. 3. Section of *Oxalis* stem showing low iron content but uniform distribution of iron in practically all tissues.

While the results obtained by chemical analyses of the plant tissues and of the extracted tissue fluids are in agreement with the results obtained by microchemical and microscopical studies of sectional areas, as here described, the former method does not, by any manner of means, give as detailed or as accurate a picture of the conditions with respect to iron and pH relations as does the latter method.

Rumex.—As will be observed from the data of table V, the pH values of the tissues across a sectional area of *Rumex* petiole are rather low, ranging from 4.4 to 4.0 for phloem to 2.8–2.4 for sclerenchyma cells. These

TABLE V

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF *Rumex* IN CROSS-SECTION OF LEAF PETIOLE

TISSUES	pH VALUES	TISSUES	pH VALUES
Cortex	4.0–3.6	Xylem	3.0–2.6
Phloem	4.4–4.0	Pith	3.8–3.4
Sclerenchyma	2.8–2.4		

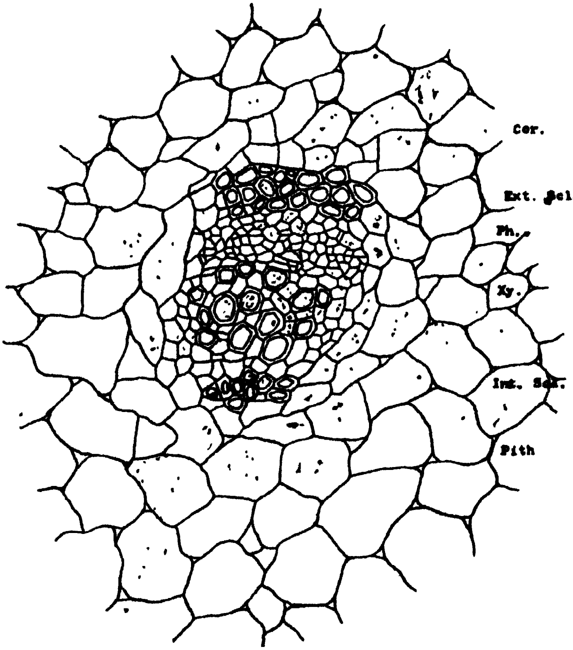

FIG. 4. Section of *Rumex* petiole showing low content of iron but somewhat uniform distribution in practically all tissues.

data are typical of those for sections taken from any part of the plant. The diagram of figure 4, like that of figure 3 for *Oxalis*, shows no accumulation of iron in any of the tissues, but indicates a fairly uniform distribution of iron in relatively small quantities throughout the cross-section.

It is thus apparent that in plants in which all the tissues show relatively low pH values, iron does not accumulate in any one tissue to a greater extent than it does in another. Furthermore, chemical analyses of the filtered tissue fluids of such plants indicate that most of the iron is in the soluble form. It is therefore to be concluded that iron migrates freely in these plants, and that there is no serious obstruction by any of their tissues to its movement from channels of translocation to the chlorophyllous cells of the leaves. Plants of this type have shown no tendency to become chlorotic from lack of iron when grown in artificial culture media containing mere traces of iron, even under experimental conditions definitely unfavorable for growth. These plants present no difficult iron problems, such as are frequently encountered in attempting to grow in artificial growth media plants in which the pH values of the tissues lie close to those representing the upper limit in the range for iron precipitation.

CORN.—A thorough study was made of sectional areas of the corn plant, for the reason that, when growing this plant in culture solutions, some difficulty was always experienced in preventing chlorosis of the leaves caused by the apparent absence of sufficient iron in the leaf mesophyll. The pH values of the composite tissue fluids extracted are relatively high. Table VI, however, shows the xylem and the cells of the bundle sheath to have relatively low pH values; while the phloem, cortex, and leaf mesophyll have high pH values.

TABLE VI

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF STEM AND LEAF OF CORN PLANT STUDIED IN CROSS-SECTION

TISSUES	pH	TISSUES	pH
Xylem (parenchyma)	4.8–4.6	Phloem	6.2–5.8
Xylem (vessels)	4.4–4.0	Cortex	6.0–5.6
Bundle sheath	4.6–4.4	Leaf mesophyll	6.0–5.6
Sclerenchyma	4.8–4.6		

In direct contrast to the conditions found in *Oxalis* and *Rumex*, the corn plant shows very heavy accumulations of iron, primarily in the bundle sheath and in cortical cells immediately surrounding it. These conditions are shown in the diagram of figure 5, which is typical of many sectional areas prepared from stems and leaves. It is significant that in both the stem and leaf of corn, small amounts of iron were always found in the vessels. This was usually observed in all the plants studied. No iron was found in cortical cells of the stem, nor in mesophyllous cells of the leaf slightly removed from the bundle region. No trace of iron was found in the phloem of the stem or leaf. Apparently iron penetrates the phloem of the leaf and stem with difficulty or not at all. The pH of this tissue is the highest of any found in corn.

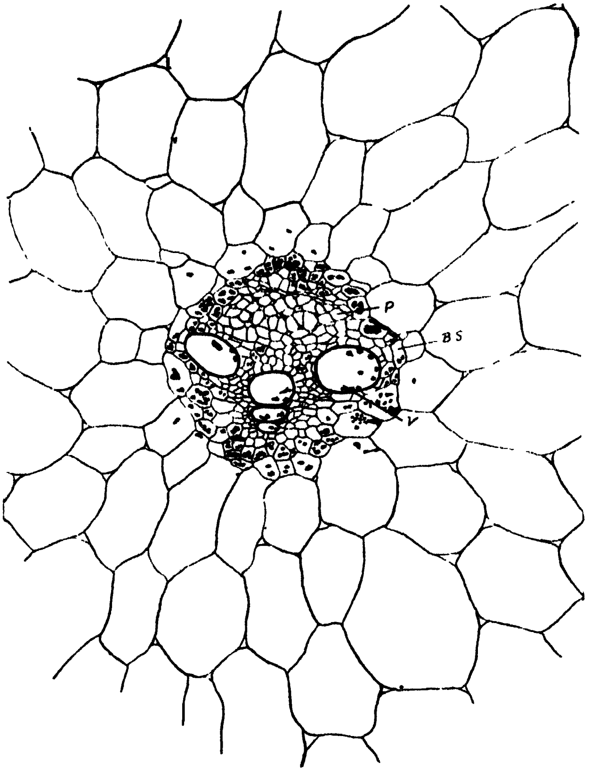

FIG. 5: Section of corn stem showing very heavy iron accumulations in the tissues of the bundle sheath.

It is remarkable to note that in a chlorotic corn leaf, chlorophyll was present only in the mesophyllous cells surrounding the bundles and those near the strip of sclerenchymatous tissue near the upper surface opposite each large vascular bundle. In the leaf blade, beginning a short distance from the midrib, the bundle and this strip of mechanical tissue approach each other rather closely, so that the spongy tissue between the two is only two or three cells in thickness. In a chlorotic leaf, these cells, as well as those near the bundle and sclerenchymatous tissue, possess chlorophyll, while those between the bundles are devoid of it. Such a condition is externally apparent to the naked eye. The course of the veins is marked by dark green lines, while the space between them is yellow or greenish yellow, depending upon the degree of chlorosis.

A study of sectional areas of chlorotic corn leaves and of green leaves brings out the fact that chlorotic leaves contain as much or more iron than do green leaves, in so far as this can be detected by microchemical methods. This is indicated also by chemical analyses of the tissues in question. In the chlorotic leaves, however, the iron is confined to the bundle regions; and from these regions it penetrates, if at all, only to the cells lying in close proximity to them or to sclerenchyma tissue. This is undoubtedly due, in large measure, to the steep pH gradient existing between the cells of the bundle sheath and those immediately to the exterior.

The root tissues of corn, as a whole, are somewhat more acid than are the corresponding stem tissues. In the roots of corn, iron in very small quantities was found somewhat well distributed, except that small accumulations were sometimes observed in the cells between the endodermis and the xylem, and in the phloem. In the prop roots, larger accumulations were observed in the phloem.

From the foregoing considerations, it is evident that in the corn plant certain tissues may offer considerable resistance to the passage of iron from the channels of translocation to the chlorophyllous cells, and such obstructions may become rather serious under slightly unfavorable growth conditions. In this plant, the morphological structure is such that the channels of iron translocation are completely surrounded by tissues with very high pH values, through which iron migrates with difficulty.

TABLE VII

APPROXIMATE pH VALUES OF VARIOUS TISSUES OF LEAF PETIOLE OF CLOVER AND SOY BEAN STEM STUDIED IN CROSS-SECTION

PLANT	TISSUES	pH	TISSUES	pH
Clover	Xylem (vessels)	4.8–4.6	Phloem	7.2–6.8
	Xylem (parenchyma)	5.6–5.4	Cortex	6.8–6.4
	Sclerenchyma ..	4.8–4.6	Pith	6.8–6.4
Soy bean	Xylem (vessels)	5.0–4.6	Phloem .	7.4–6.8
	Xylem (parenchyma).	6.6–6.2	Cortex .	7.0–6.6
	Sclerenchyma ..	5.0–4.6	Pith . ..	7.2–6.6

Clover and soy bean.—The pH data for clover and soy bean are given in table VII. It will be observed that the tissues of these two legumes, except the xylem and sclerenchyma, show relatively high pH values. In these two plants there is always a steep pH gradient between xylem and phloem, between sclerenchyma and phloem, and between sclerenchyma and cortex; so that from the xylem outward there are regions of relatively low pH which alternate with regions of high pH. The diagrams of figures 6 and 7, which represent respectively cross-sectional areas of petiole of clover and stem of soy bean, show that while iron occurs generally in the xylem tissues, it accumulates to a marked degree in the border regions between xylem and phloem and between sclerenchyma and phloem. This is particularly true of the soy bean, whose tissues show somewhat higher pH values than do the corresponding tissues of clover. Much of this accumulated iron is without doubt in the precipitated form, since chemical analyses show very high total iron for these plants and exceedingly low total iron for the filtrates of the extracted tissue fluids. Small amounts of iron were generally found also in the cortical tissues of these two species, just outside of low pH sclerenchyma. While iron was not found in this mechanical tissue,

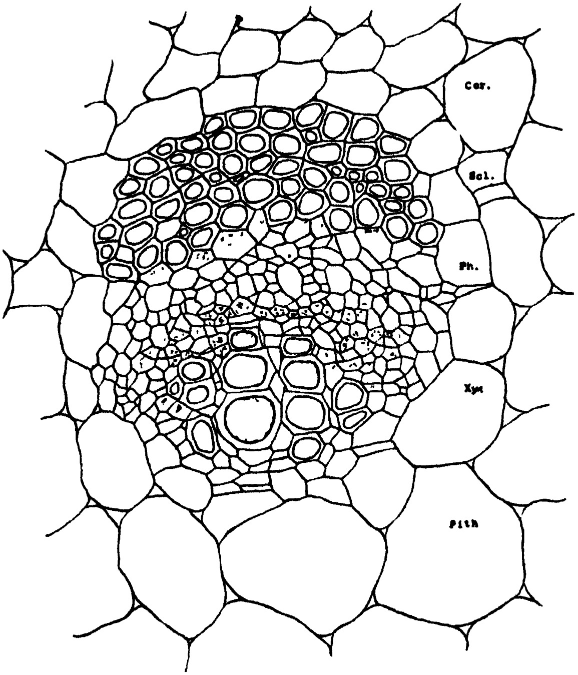

Fig. 6. Section of clover petiole showing iron accumulation in the phloem.

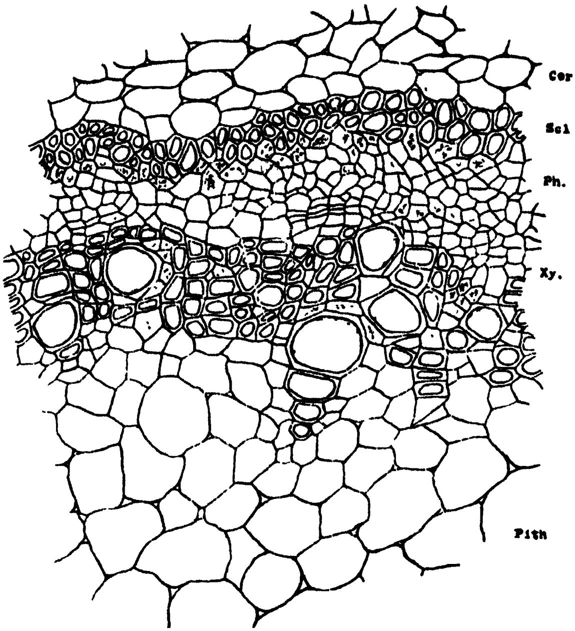

FIG. 7. Section of soy bean stem showing heavy accumulations of iron in the phloem.

except an occasional trace, it appears to have some influence in the translocation of iron into bordering tissues having much higher pH values.

POTATO.—As indicated in table VIII, the pH values of the tissues of the potato plant are very high, ranging from pH 7.6–6.6 for phloem to pH 5.0–4.6 for xylem. There is thus a steep pH gradient between xylem and phloem, with heavy accumulations of iron in the phloem, as indicated in the diagram of figure 8, representing a cross-sectional area of a leaf petiole. Small quantities of iron are also found in the vessels and scattered throughout the cells of the xylem parenchyma.

TABLE VIII

APPROXIMATE PH VALUES OF VARIOUS TISSUES OF STEM AND PETIOLE OF POTATO PLANT STUDIED IN CROSS-SECTION

TISSUES	PH	TISSUES	PH
Xylem (parenchyma)	6.6–6.2	Cortex	6.8–6.4
Xylem (vessels)	5.0–4.6	Pith	7.0–6.4
Phloem	7.6–6.8		

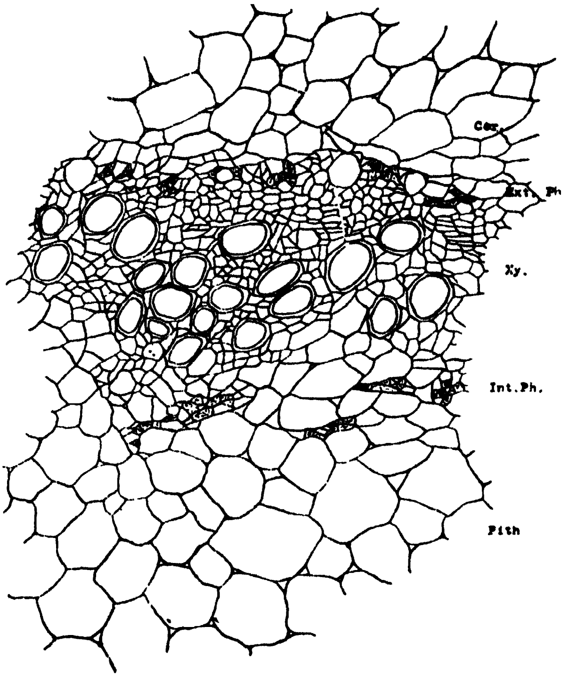

FIG. 8. Section of stem of potato plant showing heavy accumulations of iron in the external and internal phloem.

As has previously been shown, the total iron content of this plant is very high, while the iron content in the filtrate of its extracted juices is very low. It appears, therefore, that the iron accumulation in the internal and external phloem largely represents precipitated or non-functioning iron.

The potato plant, in marked contrast to the corn plant, may be grown in artificial culture media without showing such a strong tendency toward becoming chlorotic as is exhibited by the corn plant in the same medium, although the tissues of the former show considerably higher pH values than do the corresponding tissues of the latter, and in spite of the fact that the tissue fluids of the corn contain a much higher filterable iron content per gram of dry tissue than do those of the potato. It is apparent, therefore, that there is an inherent difference between these two plants with respect to the economy of iron utilization and its transfer from channels of translocation to chlorophyllous cells, for which at the present time no adequate explanation can be offered.

Discussion

A study of the tissues of different plants, and the determination of their approximate pH values, bring out the fact that, in all the plants studied, the xylem tissues always showed the lowest pH values and phloem the highest. Sclerenchymatous tissues, whenever these occurred, showed pH values which were about the same or slightly higher than those of the xylem.

In the study of iron distribution in the plants, as determined by the microchemical methods here employed, it is significant that small amounts of iron were always present in the vessels and in the xylem parenchyma, but in no instance did pronounced iron accumulation occur in these tissues. On the other hand, marked accumulations of iron, whenever these were present, usually occurred in high pH tissues, such as phloem where this bordered on the xylem or other low pH tissue. As already explained, much or all of the iron in these heavy accumulations may be regarded as being in the precipitated form, not mobile, and therefore cannot function in the metabolic processes of the plant. It is logical to conclude also that the xylem furnishes the tissues in which the upward translocation of iron occurs.

The root tissues of the plants here studied, as a whole, appeared somewhat more acid than did the corresponding tissues of the stem. From the standpoint of pH values, plants such as corn should experience no great difficulty in absorbing iron from the surrounding medium, provided that an available supply is present, since epidermis and subepidermal tissues of the roots of all the corn plants studied were relatively quite acid, and no cortical tissue of roots was found which showed pH values at or above the extreme upper limit (pH 6.0) in the range of iron precipitation as determined by PATTEN and MAINS.

The only tissues in which iron was found to accumulate in the corn roots were the cells between the endodermis and xylem and in the phloem, and these accumulations were found only occasionally and were not particularly pronounced. In all the species investigated, iron was found in small quantities in the young roots, but no accumulations were observed in these organs, the iron being rather uniformly distributed throughout the tissues.

Although iron is directly or indirectly necessary for chlorophyll production, it is apparent that if the chlorophyllous cells are comprised in a tissue with a pH reaction near or above the upper limit in the range of iron precipitation, the iron may be prevented from entering or penetrating such tissue because of precipitation, as a result of which a chlorotic condition may occur. As already pointed out, however, the range in pH values over which iron precipitation occurs in some plants is wider than that indicated by PATTEN and MAINS for inorganic systems. Hence in certain plants it is possible that iron in small quantities may penetrate tissues having pH values considerably above the upper limit (6.0) of this range, while in others it may not be possible for iron to penetrate such tissues. The former type of plant may show no tendency toward chlorosis, when subjected to a given set of conditions under which the latter may suffer severely from chlorosis owing to lack of iron in chlorophyllous cells. Two species which showed this pronounced contrast under like experimental conditions are corn and potato, the former showing a marked tendency toward chlorosis from lack of iron in chlorophyllous tissues under certain experimental conditions, while the latter showed no such tendencies under the same conditions, in spite of the fact that the potato plant contains a much lower percentage of soluble (filterable) iron than does the corn plant. The plants of both these species contain a large excess of total iron, most of which is in the insoluble (non-filterable) form.

Many agricultural plants with tissues having high pH values show these same inherent differences, for which, as has previously been pointed out, no adequate explanation can be offered at the present time on the basis of experimental evidence. It may be suggested, however, that certain organic iron compounds are able to resist the precipitating action of alkaline media, and that iron in the presence of certain organic compounds is retained in solution form in alkaline media. MARSH and SHIVE (**19**) have shown that, of the four iron compounds (ferric glycerophosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate) in solution in a culture medium at pH 6.2, no ferrous sulphate and no soluble ferric phosphate remained in solution at the end of a given experimental period; while 40 per cent. of the iron present in the same medium as ferric tartrate and 20 per cent. as ferric glycerophosphate remained in solution at the end of the period under the same experimental conditions.

REED and HAAS (**25**), in a series of qualitative tests, found that tartrates and citrates were effective in holding iron in solution in an alkaline medium. Citrates were somewhat more effective in this respect than tartrates. By testing for iron with potassium thiocyanate, they found abundant quantities in solution in a medium at pH 7.6, with sodium citrate present. FISCHER (**7**) obtained similar results using glycerol in an alkaline medium. DAKIN (**6**) mentions that this also holds true for hydroxyaspartic acid, although he gives no experimental evidence. GILE and CARRERO (**8, 9, 10**) found that, of a number of iron sources, only ferric tartrate furnished sufficient iron for rice grown in alkaline solutions. When judged by the growth of rice plants, ferrous sulphate, ferric citrate, and ferric tartrate afforded sufficient iron, when used in proper quantities in acid and neutral solutions. Many other workers have found similar results with certain organic compounds.

SMYTHE and SCHMIDT (**28**) state that those substances which possess a certain particular grouping within the molecule will hold iron as an undissociated compound. Such a combination in most cases is rather resistant to the action of alkaline solutions. They found the following classes of substances to possess the necessary grouping: hydroxymono-carboxylic acids (lactic, gluconic); dicarboxylic acids (oxalic, malonic); hydroxydicarboxylic and hydroxytricarboxylic acids (tartaric, citric); amino acids which are also hydroxy- or dicarboxylic acids (aspartic acid, serine); certain inorganic acids (phosphoric, arsenic); certain phosphorus-containing compounds (nucleic acid, glycerophosphoric acid); and certain proteins (casein, gelatin). They suggest an explanation, based on the residual charge of ions, for the manner in which the iron may be united.

It appears logical to assume that, if such compounds as those just mentioned serve to hold iron in solution in an external alkaline medium, they should serve the same purpose inside the plant, provided that they are present there. Many such compounds are known to be present in some plants, and certain ones may be entirely lacking in others. It may then be suggested as a possibility that some plants with high pH tissues may show little tendency toward chlorosis, even under somewhat unfavorable growth conditions, because they may contain certain organic solvents, in the presence of which iron in the mobile form resists the precipitating influence of high pH reactions. On the other hand, certain plants with high pH tissues may show a strong tendency toward chlorosis, because they may not contain the particular organic solvents which hold iron in solution in the tissues against the precipitating influence of high pH reactions.

It seems probable that the precipitated iron which is present in certain tissues of high pH plants may serve as a reserve iron supply, in that certain of these organic solvents may move across such tissues containing the precipitated iron, and may become effective factors in translocating some of it to tissues more remote.

Summary

1. Plants which yield composite tissue fluids having high pH values in general show very low soluble (filterable) iron content and very high total iron.

2. Plants which yield composite tissue fluids having low pH values in general show relatively high soluble iron and low total iron. In some cases nearly all of the iron in the plant samples appears in the filtered extracts.

3. The range of pH values over which iron appears to precipitate in plants of different species is wider than the corresponding range for inorganic systems.

4. Plants whose extracted tissue fluids show very high pH values, and those with tissue fluids having very low pH values, still show slight fluctuations in soluble iron content over a day and night period, which corresponds in the inverse relation to fluctuations in pH values of the tissue fluids due to variation in light intensity over the same period.

5. The highest pH values of specific tissues for each species investigated occurred in the phloem, with cortex only slightly lower; and the lowest pH in the xylem. Steep pH gradients always occurred between xylem and phloem.

6. Iron accumulations usually occurred in high pH tissues lying adjacent to relatively low pH tissues with a steep pH gradient between. It is concluded from chemical analyses that iron in these accumulations is in a precipitated form and not available for plant processes.

7. No iron accumulations were found in plants with low pH tissues throughout. In these plants the iron content is low and uniformly distributed in practically all of the tissues.

8. The xylem furnishes the main channels of iron translocation from the roots to the leaves.

New Jersey Agricultural Experiment Station,
New Brunswick, New Jersey.

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HYDROGEN-ION CONCENTRATION AND IRON CONTENT OF TRACHEAL SAP FROM GREEN AND CHLOROTIC PEAR TREES

J. OSERKOWSKY

(WITH TWO FIGURES)

Introduction

The occurrence of chlorosis on muck soils to which lime was added was found by LOEHWING (4) to be correlated with low acidity of the sap of plants growing on these soils, as compared with the H^+ concentration of the sap from similar plants grown on unlimed soils. It was held that on the limed soils the iron failed to reach the leaves in sufficient amounts, because the iron was precipitated in the roots and the stems, on account of the low acidity of the sap in these parts. MARSH and SHIVE (5) observed that the iron content of stems of chlorotic soy beans was higher than that of stems from green plants, while the iron content of the chlorotic leaves was lower than that of green leaves.

The work just referred to was conducted with annual plants. It has been observed, however, that chlorotic leaves from fruit trees may contain as much or more iron than green leaves (7). Further, MILAD (6) and WALLACE (8) did not find a significant difference between the iron content of bark or wood of stems and roots from chlorotic and green trees. These findings indicate that chlorosis cannot be explained solely on the assumption that iron fails to reach the leaves in sufficient amount for normal development of chlorophyll. If iron moves to the leaves in the tracheal sap, then the question arises, is the H^+ concentration of the tracheal sap sufficiently high to prevent the absorbed iron from precipitating in the stem before reaching the leaves? The present work was undertaken in an attempt to answer this question.

Material and methods

Three series of branches of Hardy pear trees were collected from an orchard situated in the Santa Clara Valley in California. The soil in this locality is rich in lime, as a consequence of which "lime-induced chlorosis" is common. Practically all the trees in this orchard suffered from chlorosis, which was found to be curable only by administration of iron salts to the trees. One series comprised branches from normally green trees; another series comprised branches from chlorotic trees which were treated with iron citrate, as a consequence of which they developed deep green leaves; and a third series comprised chlorotic branches. A fourth series of samples was taken from another orchard situated also in the Santa Clara Valley, but in which all the trees were healthy and green.

The samples were collected at different times of the day, but all branches collected at any date were taken at the same time of the day, in order to eliminate the effect of possible diurnal differences in pH. As soon as the branches were severed from the trees, the leaves and twigs were removed in order to prevent loss of water by transpiration. The branches were then removed to cold storage (36° F.) over night, after which the tracheal sap was extracted according to the method of BENNETT, ANDERSSEN, and MILAD (**3**). The pH of the sap obtained from each branch was determined separately by the quinhydrone electrode. The results obtained with this electrode were in fair agreement with those secured by the colorimetric method. Each of the pH values in table II represents an average of four to six branches.

While it is possible that the pH of the tracheal sap underwent some changes during storage, experience in this laboratory had shown that the magnitude of these changes hardly exceeds a few tenths of a pH unit. Such changes are considered insignificant, since they are of the same order of magnitude as differences in pH between branches of the same series collected at the same time. The variation in pH of the tracheal sap of such samples can be gauged from table I, in which are presented the pH values of sap obtained from thirteen branches collected on April 1 at the same hour of the day.

TABLE I

H^+ CONCENTRATION OF EXTRACTED TRACHEAL SAP OF CHLOROTIC PEAR BRANCHES

	NO. OF BRANCH													
	1	2	3	4	5	6	7	8	9	10	11	12	13	AVERAGE
pH . . .	5.52	5.49	5.45	5.34	5.40	5.14	5.45	5.47	5.47	5.60	5.52	5.55	5.72	5.48

The determination of iron in the tracheal sap was carried out as follows. Aliquots of the sap were evaporated in porcelain crucibles, ashed, dissolved in aqua regia, evaporated to dryness, dissolved in 0.5 N NHO_3, and made up to a definite volume. To an aliquot of this solution KCNS, and ethyl-acetate or amyl-alcohol, were added, the mixture shaken, allowed to stand for a few minutes, and the iron in the amyl-alcohol or ethyl-acetate phase determined colorimetrically. Determinations of iron in blanks were made, and the results given in table II corrected accordingly.

Results and discussion

Figure 1 shows that the H^+ concentration in the tracheal sap is higher at the beginning of the season than later. The period of high acidity lasts in green trees for about six to eight weeks, while in chlorotic trees it is

TABLE II

CONCENTRATION OF H· AND IRON IN THE TRACHEAL SAP OF GREEN AND CHLOROTIC BRANCHES FROM HARDY PEAR TREES

DATE OF COLLECTING SAMPLES		pH OF TRACHEAL SAP				IRON IN TRACHEAL SAP (MG. FE PER LITER)			
		SERIES OF SAMPLES							
		GREEN BRANCHES FROM HEALTHY TREES IN CHLOROTIC ORCHARD	GREEN BRANCHES FROM TREES TREATED WITH IRON	CHLOROTIC BRANCHES	GREEN BRANCHES FROM HEALTHY ORCHARD	GREEN BRANCHES FROM HEALTHY TREES IN CHLOROTIC ORCHARD	GREEN BRANCHES FROM TREES TREATED WITH IRON	CHLOROTIC BRANCHES	GREEN BRANCHES FROM HEALTHY ORCHARD
March	12			6.65					
"	16			6.92				-0.14	
"	31		5.59	5.69			10.0		0.22
April	1				5.76				0.36
"	5	5.64				0.29		0.087	
"	6				5.79				
"	21				5.76				0.61
"	26	5.72		5.40		0.53		0.49	
May	1					0.17	0.21	0.10	0.19
"	28	5.69	5.72	6.27	5.94				
June	15	5.84	5.94	6.19	5.85		0.34	0.17	0.20
July	6				6.50				
"	9	6.18	6.18	6.17		0.047		0.11	
"	11				6.29				
Aug.	22	6.69	6.87	6.68	6.90	0.099	0.36	0.13	0.17
Sept.	19	6.72		6.79	6.89	0.075	0.10	0.037	0.24
Oct.	21					0.33			
"	30	7.12	7.04	7.00	7.20			0.015	0.075
Dec.	18					0.22			
"	28						0.15		0.13
Feb.	7					0.097		0.16	0.12

shorter. The same difference between chlorotic and green trees was observed also in Bartlett pear trees grown in the Santa Clara Valley. This difference in the H^+ concentration is correlated, presumably, with growth activity of the trees.

Except for the difference in the duration of the period of high acidity, no significant difference exists between the pH of the tracheal sap of green and chlorotic trees. The H^+ concentration of the sap from chlorotic branches may be equal to or greater than that of green branches collected at the same date and at the same hour of the day. The concentration of iron in the tracheal sap of branches from the healthy orchard is higher practically throughout the season than that of the chlorotic branches. The "healthy orchard" referred to was situated in a lime soil, and, although the trees in this orchard could not be classed as chlorotic, there were indications in the form of slight traces of chlorosis that they were on the verge of becoming so. Additional data, not reported in this paper, indicate that the iron content of the tracheal sap of branches from Bartlett pear trees grown in non-calcareous soil is, on the whole, higher than that of branches from trees grown in lime soils.

It should be borne in mind that the extracted tracheal sap is in equilibrium with the atmospheric air; while in the plant the tracheal sap is in contact with the gases of the cellular and intercellular spaces, which are in all probability much richer in CO_2 than in air. Since the tracheal sap possesses a weak buffer capacity (1), it is very likely that *in situ* its H^+ concentration may be materially affected by the CO_2 concentration of the internal gas. The pH of the extracted sap may not, therefore, represent

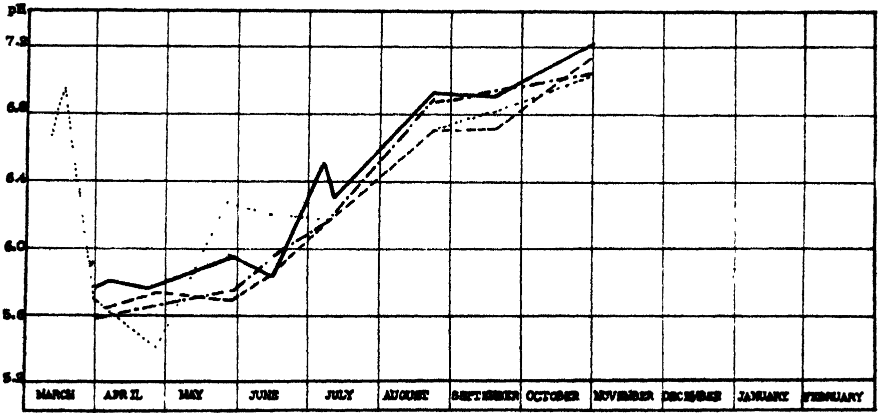

FIG. 1. Seasonal changes of H^+ concentration in tracheal sap of branches from Hardy pear trees. Legend: ———— green branches from healthy orchard; –.–.–. green branches from trees treated with iron; .... chlorotic branches; – – – – – green branches from healthy trees in chlorotic orchard.

the true H^+ concentration of the tracheal sap in the plant. Since, however, it seems that most of the CO_2 present in the sap escapes from it after its extraction, it is believed that the amount of iron in the extracted sap is substantially the same as that of the tracheal sap *in situ.*

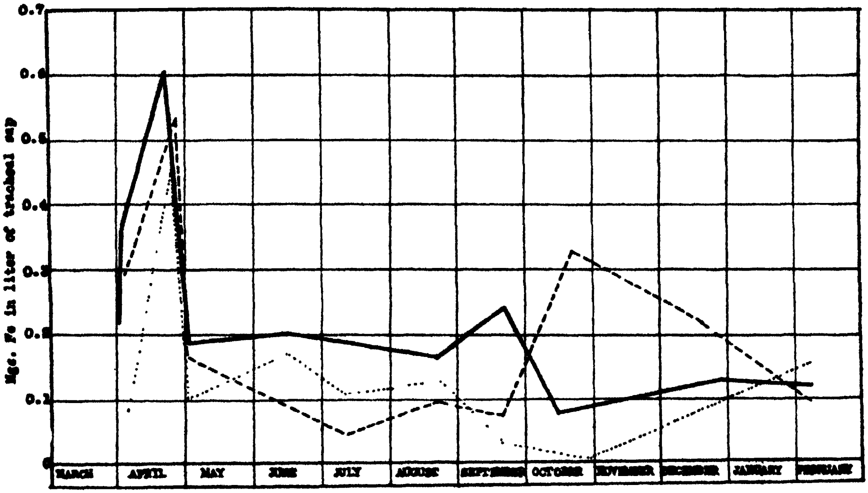

FIG. 2. Seasonal changes in concentration of iron in tracheal sap from branches of Hardy pear trees. Legend: ———— green branches from healthy orchard; .... chlorotic branches; – – – – – green branches from healthy trees in chlorotic orchard.

The data relating to the concentration of iron in the tracheal sap are presented in table II and figure 2, from which it is seen that, at the beginning of the growth season, the concentration of iron increases very rapidly and then falls abruptly. This rise and fall takes place during the swelling of the buds and blossoming; it lasts three to four weeks. It yet remains to determine what portion of the iron in the tracheal sap during this period is derived from the stored reserves in the plants.

The concentration of iron in the sap fluctuates during the rest of the season, but remains on the whole at a lower level. In the sap from trees which were treated with iron[1] during the winter, before growth started, the concentration of iron was higher during the first part of the season than it was in sap from untreated trees, which was to be expected.

The fact that concentration of iron in the tracheal sap falls shortly after the beginning of the growth season is no indication that the rate at which iron is absorbed by the tree decreases. Indeed the rate of accumulation of iron in leaves is practically constant throughout the growing season.

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[1] The iron was administered to the trees in the form of iron citrate powder introduced at the bottom of the trunk. For details concerning the method of treatment, see BENNETT (2).

The decrease in iron concentration in the tracheal sap may be due, at least partly, to the increased rate of transpiration of the tree as the area and number of leaves increase with the advance of the season.

It has just been shown that both the H^+ and iron concentrations of the tracheal sap are higher early in the season than later. On examination of figures 1 and 2, it may be observed that the H^+ and the iron concentrations for any series of samples fail to show a consistent correlation. Additional data, not presented in this paper, show that the iron concentration in the tracheal sap of pear trees grown in non-calcareous soil is higher than that of sap from green or chlorotic trees grown in lime soils. This condition holds true throughout the growing season, notwithstanding the fact that the pH of the tracheal sap of the trees from the non-calcareous soil may be higher than that of trees grown in calcareous soils. The concentration of iron in the tracheal sap of pear trees is very low, however, and may fall short of the saturation point of iron consistent with the existing pH of the sap. In view of this fact, the extent to which the concentration of iron in the tracheal sap of pear trees may be subject to regulation by the pH of the sap yet remains to be determined. It does not seem probable that, in the samples dealt with in this paper, pH was limiting the concentration of iron in the tracheal sap. It is plausible, however, that in those plants in which iron is more abundant in the tracheal sap, its concentration may be limited by the H^+ concentration of the sap.

Conclusions

1. The concentrations of H^+ and iron in the tracheal sap of pear trees reach a high level at the beginning of the growing season; but the period at which the concentration of iron is high is shorter than the period of low pH of the tracheal sap.
2. No significant difference exists between the H^+ and the iron concentrations in the extracted tracheal sap of green and chlorotic branches from the same orchard; the concentration of iron in the tracheal sap from chlorotic trees may be higher than that from green trees.

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Division of Plant Pathology,
University of California.

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USE OF EXPRESSED SAP IN DETERMINING THE COMPOSITION OF CORN TISSUE[1]

J. D. SAYRE AND V. H. MORRIS

(WITH FIVE FIGURES)

The composition of sap expressed from plant tissues has been much studied during recent years in various physiological investigations. Most of the investigators, however, have not attempted to relate the composition of the expressed sap to that of the original tissue. In a recent paper on the use of expressed sap in physiological studies of corn (**6**), it was shown that the sugar content of corn tissues could be estimated from the analysis of the expressed sap with a fair degree of accuracy. Certain refinements in technique and calculation have been applied to this method of determining the sugar content of corn tissues with more satisfactory results. The determination of the composition of corn tissues with respect to other constituents by the analysis of expressed sap also has been investigated. The results of these studies are presented in this paper.

The apparatus used for expressing the sap from corn tissues and the details of the general technique were similar in all essentials to those described previously (**6**).

Determining the composition of the tissue from analysis of the expressed sap

Moisture content of the tissue as determined by this method represents the sum of the moisture in the press cake plus the moisture in the expressed sap (**7**). The moisture in the press cake was determined by drying in an air oven, and that of the expressed sap by the refractometer method of GORTNER and HOFFMAN (**3**). The total solids content of sap from leaf tissue determined by the refractometer was consistently higher than that determined by vacuum oven drying. The relation between the methods, based on determinations made at regular intervals during the season, is shown in figure 1. This regression may be used to correct the determinations by the refractometer method in sap from leaf tissue of corn. No correction in sap from stem tissue is necessary.

It was shown in the previous paper (**6**) that the total sugar content of corn tissue can be calculated from an analysis of expressed sap with a fair degree of accuracy. In this calculation the assumption is made that the

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[1] Based on investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

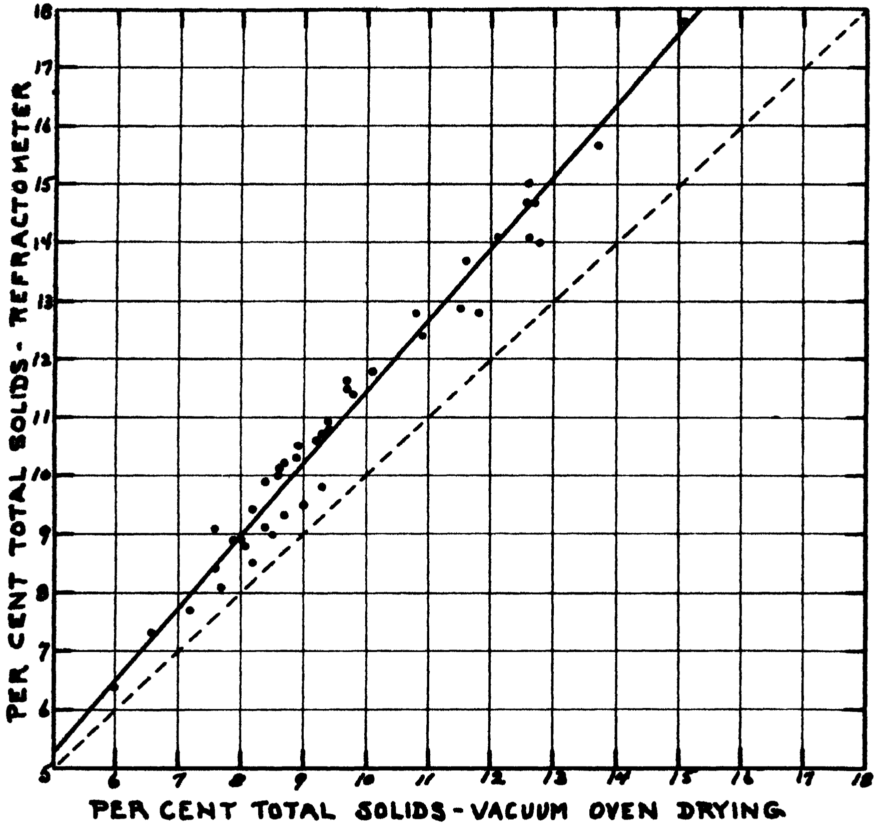

FIG. 1. Comparison of total solids content of expressed sap from corn leaves determined by the refractometer method and by vacuum oven drying.

composition of the sap expressed is similar in sugar concentration to the sap remaining in the press cake. In calculating the sugar content of the tissue, no correction was made for the specific gravity or the total solid content of the sap; consequently the results were slightly lower than those obtained by the standard alcoholic extraction method. Additional determinations were made during the summer of 1930, correcting for these facts.

In order to determine the sugar content of corn tissue from an analysis of the expressed sap, it is necessary to know the moisture content of the tissue and the sugar content and moisture content of 100 ml. of the expressed sap. From these data, the percentage of sugar in the tissue is calculated by the following formula:

$$\begin{array}{c}\text{Per cent. of sugar}\\ \text{in tissue}\end{array} = \frac{\begin{array}{c}\text{Grams of sugar in}\\ \text{100 ml. sap}\end{array} \times \begin{array}{c}\text{total moisture content}\\ \text{of tissue, grams}\end{array}}{\text{Grams of moisture in 100 ml. sap}}$$

A method essentially similar to this has been used by Mason and Maskell (4).

The sugar analyses were made on 50 ml. of sap preserved by boiling in alcohol and the results expressed in grams of sugar in 100 ml. of the sap. The grams of moisture in 100 ml. of sap were obtained by subtracting the corrected total solids, as determined by the refractometer, from the weight of the sap, determined through its specific gravity.

A comparison of the results obtained by the sap expression method and the alcoholic extraction method for determining sugar in corn tissue was made on a series of 24 samples, comprising leaf, stem, and tassel tissue, taken during the period from July 7 to September 8, 1930. Free reducing sugars, sucrose, and total sugars were determined on each sample by both methods. The determinations, entered in columns a and b (table I), under each method, represent single determinations on duplicate aliquots from each sample of tissue, expressed on a green weight basis.

The two methods, sap expression and alcoholic extraction, were compared with regard to: (1) agreement in determining the mean sugar content of all the samples; (2) agreement in determining the sugar content of different kinds of tissue in varying stages of development; and (3) the relative precision of the methods in determining the sugar in duplicate samples. The mean free reducing sugar content of the 24 samples was 2.618 per cent. by the sap expression method and 2.656 per cent. by the alcoholic extraction method; the mean sucrose contents were 1.660 and 1.711 per cent. respectively; and the mean total sugar contents were 4.359 and 4.445 per cent. The significance of the differences between these pairs of mean values was tested, using Fisher's (1) formula, $t=\frac{x}{s}\sqrt{n}$, and the probability tables of Student (8). The differences were such as might be due to chance, the odds not exceeding 14:1 in any comparison. It may be concluded, therefore, that the mean sugar contents of all the samples, as determined by the sap expression and by the alcoholic extraction methods, are in good agreement.

The product moment coefficients of correlation were 0.960, 0.993, and 0.996 for free reducing sugars, sucrose, and total sugars respectively. These are high enough to show that the methods are equally satisfactory over the entire range included.

The standard errors for the sap expression and alcoholic extraction methods were: free reducing sugars, 0.089 and 0.108 per cent.; sucrose content, 0.072 and 0.110 per cent.; and total sugars, 0.092 and 0.142 per cent. In each case the standard error for the sap expression method was less than that for the alcoholic extraction method. It may be concluded from these results that the sugar content of corn tissue can be determined by the sap

TABLE I

COMPARISON OF SAP EXPRESSION AND ALCOHOLIC EXTRACTION METHODS OF SUGAR DETERMINATION

		FREE REDUCING SUGARS				SUCROSE				TOTAL SUGARS			
		EXPRESSED SAP		ALCOHOLIC EXTRACTION		EXPRESSED SAP		ALCOHOLIC EXTRACTION		EXPRESSED SAP		ALCOHOLIC EXTRACTION	
		a	b	a	b	a	b	a	b	a	b	b	b
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
July 7	Leaves	0.93	0.91	0.91	0.92	0.59	0.59	0.62	0.72	1.56	1.54	1.58	1.70
" 16	Leaves	1.48	1.51	1.47	1.40	0.81	0.86	1.05	1.12	2.33	2.43	2.57	2.57
	Stems	3.83	3.88	3.86	3.93	0.83	0.77	0.85	0.77	4.71	4.69	4.76	4.74
	Tassels	2.55	2.53	2.64	2.98	0.90	0.81	0.76	0.87	3.49	3.38	3.44	3.50
" 18	Leaves	2.39	2.41	2.24	2.24	0.09	0.02	0.00	0.06	2.49	2.43	2.23	2.30
	Leaves	2.09	2.48	2.09	2.04	0.40	0.17	0.26	0.53	2.66	2.67	2.36	2.60
" 22	Leaves	1.58	1.47	1.27	1.27	0.45	0.59	0.95	0.95	2.05	2.11	2.27	2.27
	Lower stems	3.81	3.94	3.63	3.63	0.26	0.37	0.76	0.57	4.19	4.20	4.43	4.23
" 29	Leaves	2.15	2.37	1.82	1.81	0.61	0.78	0.85	0.72	2.80	3.19	2.72	2.57
	Rolled leaves	2.65	2.42	3.00	3.19	0.92	0.93	1.13	1.07	3.62	3.39	4.19	4.32
	Tassels	2.77	2.93	2.74	2.46	0.88	0.99	1.04	0.99	3.71	3.97	3.83	3.50
" 31	Leaves	2.39	2.39	2.86	2.75	0.31	0.28	0.53	0.04	2.72	2.68	3.42	2.79
	Lower stems	4.88	4.92	4.87	4.85	1.16	1.13	1.24	1.15	6.11	6.11	6.18	6.06
Aug. 5	Leaves	1.94	1.97	1.74	1.74	0.89	0.76	0.91	0.95	2.88	2.76	2.70	2.74
	Lower stems	4.25	4.37	4.14	4.14	2.40	2.40	2.36	2.39	6.77	6.90	6.62	6.66
" 11	Leaves	1.90	1.83	2.00	2.04	1.55	1.57	1.16	1.20	3.53	3.48	3.22	3.30
	Lower stems	3.90	3.86	4.04	4.36	4.30	4.30	4.39	4.41	8.42	8.38	8.66	9.00
" 12	Leaves	2.26	2.27	2.87	3.00	1.22	1.14	0.68	0.50	3.55	3.46	3.59	3.53
" 18	Leaves	2.17	2.17	2.10	2.28	0.98	0.95	0.89	1.03	3.20	3.17	3.22	3.18
	Lower stems	3.56	3.44	3.80	3.86	6.33	6.48	6.45	6.47	10.02	10.04	10.54	10.50
" 25	Leaves	1.34	1.41	1.48	1.42	1.21	1.14	1.05	1.07	2.61	2.61	2.58	2.54
	Lower stems	3.59	3.63	4.02	4.10	5.68	5.72	5.55	5.55	9.57	9.64	9.86	9.94
Sept. 8	Leaves	1.92	1.84	1.44	1.34	1.16	1.25	1.56	1.65	3.14	3.16	3.08	3.08
	Lower stems	2.21	2.17	2.38	2.34	5.97	5.80	6.02	6.29	8.46	8.28	8.68	9.00
	Mean	2.618		2.656		1.660		1.711		4.359		4.445	

expression method at least as accurately as by the alcoholic extraction method.

Relation between composition of successive portions of expressed sap and composition of the tissue from analysis of the expressed sap

It was desirable to analyze successive portions of expressed sap to check the uniformity of sugar content and also to determine other substances in the successive portions. The sap expressed from a sample of ground tissue was collected, 10 ml. at a time, as it came from the press cage, and each portion analyzed separately for total solids by the refractometer and by vacuum oven drying, for total sugars, total nitrogen, nitrate nitrogen, total phosphorus, and inorganic phosphorus.

The total solids, as determined by the refractometer and by vacuum oven drying, and the total sugars are shown in figure 2. Total solids decreased in successive portions of the sap, vacuum oven drying giving values

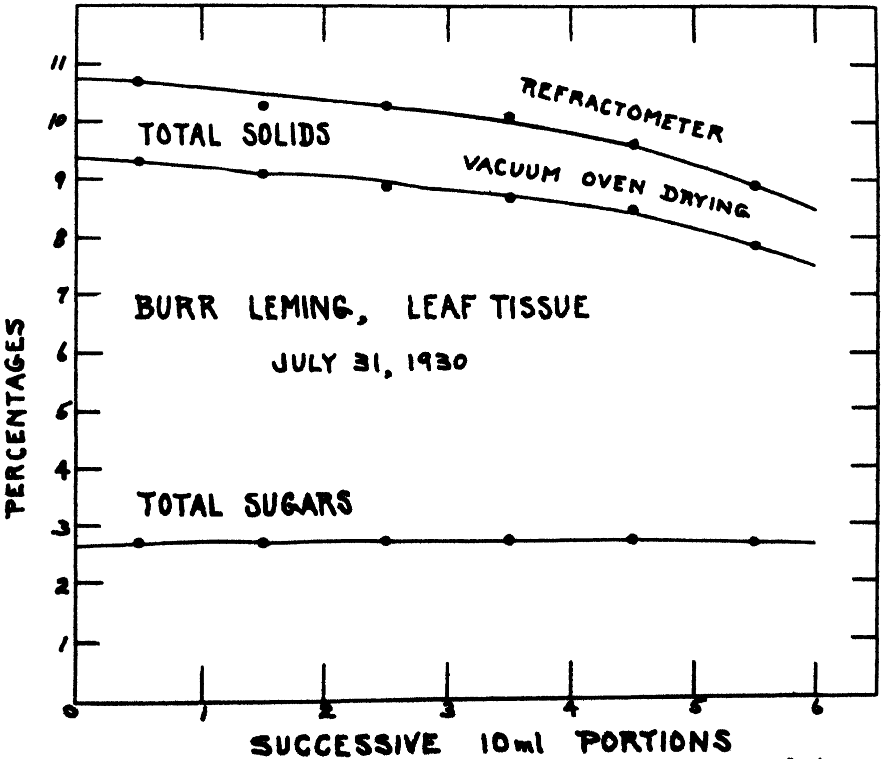

FIG. 2. Total solids, determined by refractometer and by vacuum oven drying, and total sugars in successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

similar to those obtained by the refractometer but uniformly lower. Total sugars were similar in each successive portion of the sap. Total solids in the expressed sap were composed of water-soluble and water-miscible materials from the tissue. The total solids in the first portions represent some material from the broken cells in the outer part of the mass of ground tissue in the press cage. The decrease in these materials in later portions of the expressed sap is probably due to the filtering action of the mass of tissue itself. Sugars, being in true solution in the water of the tissue, are not removed by this filtering action. Since the total sugars are constant in each successive portion of the sap, it is assumed that they are also contained in the water which remains in the press cake in this same proportion.

The total nitrogen, nitrate nitrogen, total phosphorus, and inorganic phosphorus content of the successive portions are shown in figures 3 and 4. Total nitrogen and total phosphorus decreased in each successive portion of the sap; while nitrate nitrogen and inorganic phosphorus were practi-

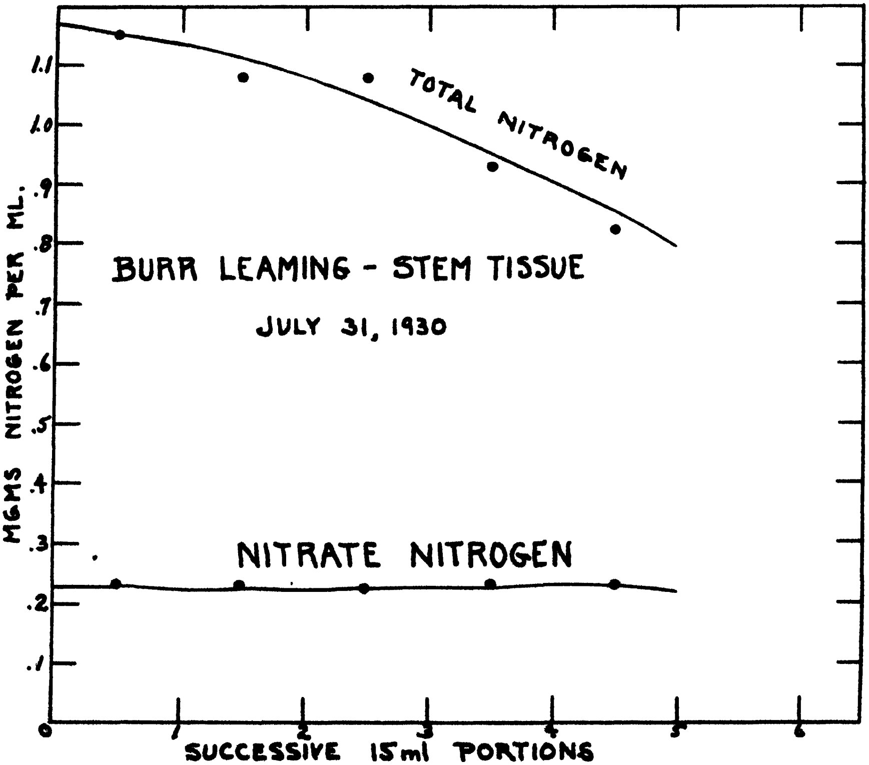

FIG. 3. Total phosphorus and inorganic phosphorus in successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

cally constant in the successive portions. These results, including those obtained on sugars, indicate that when tissue is ground and the sap removed by pressure as outlined, those substances in true solution are constant in the successive portions of the sap. It follows that the composition of the tissue with respect to such substance can be calculated from analysis of the expressed sap.

A comparison of the nitrate nitrogen content of corn tissue as determined by the sap expression and the alcoholic extraction methods has been reported by GERDEL (**2**), who found excellent agreement between the two methods.

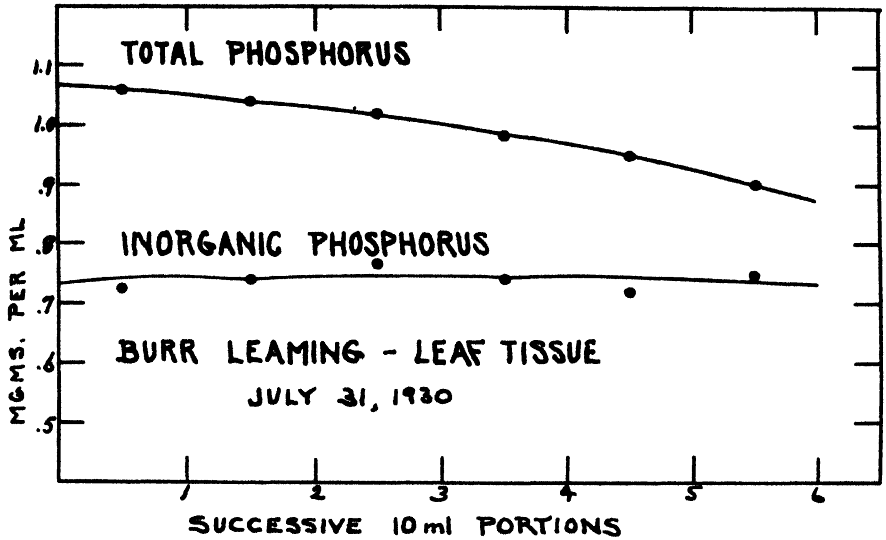

FIG. 4. The nitrogen and nitrate nitrogen in successive portions of sap expressed from stem tissue of Burr-Leaming corn.

The conductivity, freezing point, pH, and buffer action of successive portions of expressed sap were determined. The results (fig. 5) show that there was very little change in the composition of the successive portions in these properties, with the exception of the buffer action. Similar results were reported by NEWTON, BROWN, and MARTIN (**5**), in expressed sap from wheat plants.

Since the freezing point, conductivity, and pH of successive portions of sap are similar, it may be assumed that they are also similar in the sap remaining in the press cake. It is impossible, however, to tell what has happened during the grinding of the tissue (necessary to obtain a satisfactory sample of expressed sap), and thus nothing can be concluded concerning the conductivity, freezing point, or pH of the unaltered tissue. Until more satisfactory methods of measuring these properties in the living tissue are devised, no conclusions can be reached in this connection.

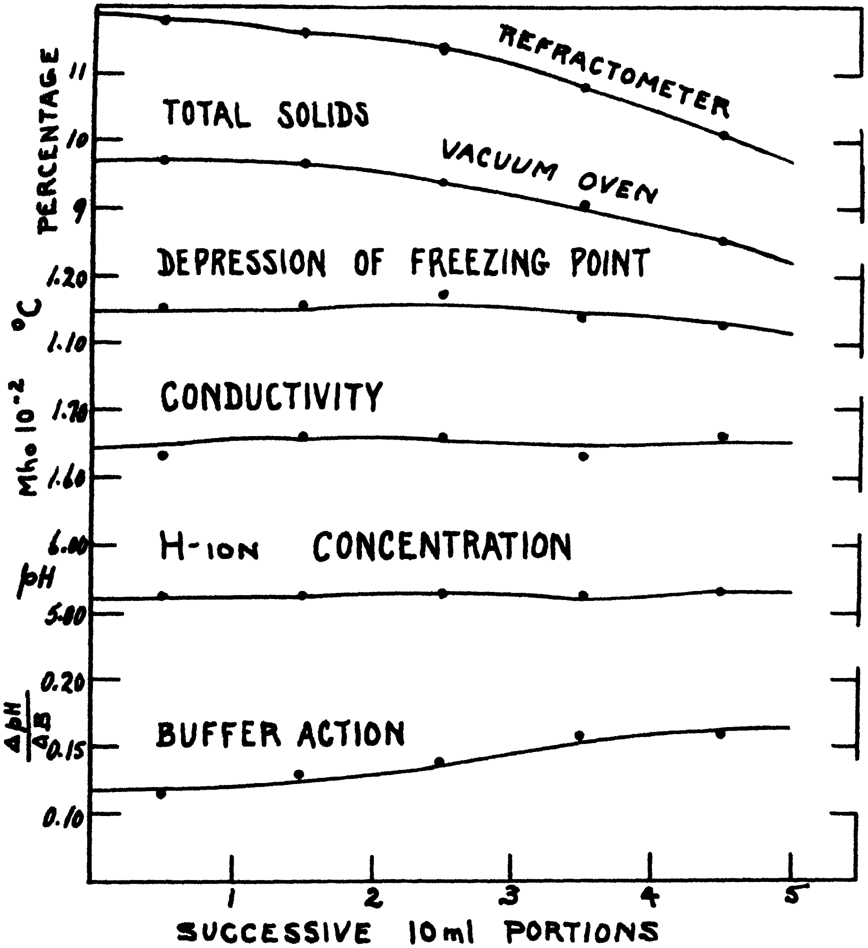

FIG. 5. Total solids, by refractometer and by vacuum oven drying, depression of freezing point, conductivity, H-ion concentration, and buffer action of successive portions of sap expressed from leaf tissue of Burr-Leaming corn.

Effect of varying size of samples, pressure, and preliminary treatment of tissue on determination of composition of the tissue by analysis of expressed sap

In the experiments reported above, a 100-gram sample of ground corn tissue, subjected to a pressure of 5000 pounds per square inch for 5 minutes,

TABLE II

AMOUNT OF SAP OBTAINED, TOTAL SOLIDS, AND PER CENT. OF SUGARS IN THE TISSUE CALCULATED FROM EXPRESSED SAP ANALYSIS UNDER DIFFERENT TREATMENTS AND CONDITIONS OF OBTAINING SAP

VARIETY	TISSUE	SAP FROM 100 GRAMS OF TISSUE	TOTAL SOLIDS IN SAP	TOTAL SUGARS IN TISSUE	REDUCING SUGARS IN TISSUE	SUCROSE IN TISSUE	SIZE OF SAMPLE	PRESSURE PER SQUARE INCH	TREATMENT OF TISSUE
		gm.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*gm.*	*lb.*	
Burr-Leaming	Leaf	62.0	9.7	2.98			25	10000	Ground
"	"	59.8	9.6	3.00			50	10000	"
"	"	57.3	9.4	2.98			100	10000	"
"	"	55.5	9.3	2.94			150	10000	"
"	"	54.7	9.4	2.90			200	10000	"
Burr-Leaming	Leaf	55.7	10.3	2.57			100	1000	Ground
"	"	59.4	10.5	2.55			100	5000	"
"	"	59.4	9.7	2.45			100	10000	"
"	"	60.6	9.9	2.57			100	14000	"
Burr-Leaming	Stem	79.4	12.4	9.01			100	1000	Ground
"	"	80.8	12.4	8.94			100	5000	"
"	"	82.2	12.4	8.99			100	10000	"
"	"	82.2	12.4	8.94			100	14000	"
Burr-Leaming	Leaf	57.5	9.4	2.56	1.43	1.07	100	10000	Ground
"	"	57.5	9.1	2.55	2.31	0.24	100	10000	Ground and frozen
"	"	...	..	2.56	1.45	1.05	100	...	Alcoholic extraction of tissue
Burr-Leaming	Stem	80.9	10.7	9.11	2.33	6.49	100	10000	Ground
"	"	81.8	10.4	9.27	2.40	6.53	100	10000	Ground and frozen

was used as the unit on which the measurements were based. It was desirable to see what effect different treatments, size of samples, and pressure would have on the results. Data which were obtained by using different sized samples in the press cage, other conditions remaining the same, are given in table II. These data show that, although the proportion of sap expressed depends on the size of the sample, a greater percentage being removed from the smaller samples, the sugar content of the tissue calculated from the analysis of the expressed sap is practically the same for each sized sample. The data, when the pressure was varied, are also given. Pressures of over 5000 pounds per square inch did not remove proportionally larger amounts of sap, and the sugar content of the sap obtained from tissue under higher pressures is not affected. Since much more trouble is encountered in using higher pressure, 5000 pounds is recommended as a convenient standard.

In some of the experiments reported by other investigators, plant tissues were frozen before expressing the sap. In a former paper (**6**) it was noted that freezing corn tissue did not result in a very much larger yield of sap than grinding; thus the latter operation was adopted as being more convenient. The possibility that freezing might change some of the properties of the sap also was considered. Some evidence on this point has been obtained in studying the effect on sugar content of freezing ground tissue at –25° C. The results are shown in table II. The amount of sap obtained and the total solids in the sap from the frozen and unfrozen tissue are not importantly different. The total sugar contents of the tissue, as estimated from the expressed sap from the frozen and unfrozen tissue, also agree well with each other and with that determined by alcoholic extraction. The sucrose in the leaf tissue, on the other hand, was much lower after freezing, indicating that hydrolysis had occurred. Similar results were obtained a number of times. Slight hydrolysis occurred in the stem tissue also, but not as much as in the leaf tissue. Total sugars, therefore, can be obtained from tissue which has been frozen, but the true sucrose content of leaf tissue can be obtained only from tissue not frozen. These results are in accord with those of MASON and MASKELL (**4**), who found an apparent inversion of sucrose in the sap of cotton leaves after freezing at –16° C. for 24 hours.

Discussion

On the basis of the results reported, it appears that when corn tissues are ground and pressed under the conditions described, the first portions of the expressed sap are obtained, for the most part, from the broken cells in the mass of ground tissue and, consequently, there is included a certain amount of colloidal protoplasmic material. As the pressure is increased the mass of tissue is compressed and begins to exert a filtering action, filtering out the larger colloidal particles. As the pressure is increased further the sap, which now is expressed for the most part from unbroken cells, is filtered to a still greater extent, so that the last portion contains a noticeably smaller amount of colloidal material than the first portion.

That this is true is indicated by the fact that the determinations which included colloidal materials, as well as those in true solution, such as total solids, total nitrogen, and total phosphorus, showed a progressive decrease with each successive portion. Materials in true solution, such as sugars, nitrates, inorganic phosphates, and such measurements as conductivity, freezing point depression, and H-ion concentration, were constant in the successive portions of the sap.

Since there is no reason to believe that grinding of the tissue and subsequent pressing out of the sap has influenced the relationship between these solutes and the solvent to any extent, it may be assumed that the concentration of the sap which has been expressed is similar to that which is retained in the press cake so far as the concentration of the solutes is concerned. It follows then that from determinations of the concentration of any solute in the solvent in the expressed sap and the total quantity of the solvent in a given sample of the tissue, the total quantity of the solute in the tissue can be calculated.

Summary

1. The procedure for calculating the total sugar content of corn tissue from analysis of expressed sap is described in some detail.

2. The total sugar, reducing sugars, and sucrose content of corn tissue can be calculated from analysis of the expressed sap just as accurately as they can be determined by alcoholic extraction of the tissue.

3. The total sugar content is not affected by the size of the sample, the pressure used, nor the preliminary treatment.

4. The sucrose content of corn leaf tissue is decreased by freezing the tissue but is not affected by grinding it.

5. It is concluded that the composition of corn tissue with respect to any constituent in true solution in the sap can be determined from an analysis of the expressed sap.

The writers take pleasure in acknowledging the helpful suggestions of F. D. Richey, Division of Cereal Crops and Diseases, Bureau of Plant Industry, during the course of these studies.

Ohio Agricultural Experiment Station,
Wooster, Ohio.

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CHANGES IN THE COMPOSITION OF THE GARDEN PEA AFTER HARVEST

H. A. Jones and C. S. Bisson

Introduction

In this paper data are presented showing some of the changes that occur in the fruit of the garden pea after harvest, when held at different temperatures. The object of this study was to determine the changes in at least a few of the components that were thought to be so altered after harvest as to affect the edible quality. In this study the changes in only a few of the carbohydrates were determined, but no doubt a large number of other chemical as well as structural changes occur that are to some extent responsible for the rapid loss of edible quality when held under certain conditions after harvest.

Experimental procedure

The variety of pea used was the Dwarf Telephone. This is a large-podded wrinkled pea, and is the one used in the Imperial Valley for shipping in the early spring. The work was started in 1925, and the material studied that year was harvested from the same field and from the same vines as used for the study of pea development previously reported (**1**). Open flowers were labeled on April 9, 1925, and the fruit of these tagged flowers was in the best stage for harvest on May 9. The fruit was harvested between 7: 30 and 8: 00 A. M. and placed in storage at once at 0, 5, 14, 25, and 35° C. In 1925 small chambers were used for storage at 25 and 35° C. In these the rapid loss of water from the fruit caused a high relative humidity and the surface of the pods remained moist. The combination of high humidity and high temperature made a favorable environment for bacterial activity and the pods started to decay after two days at 35° C. and after three days at 25° C. For this reason the data for these two high temperatures were discarded. During the entire storage period at both 0 and 5° C. the fruit remained green and turgid.

The work was repeated again in 1927 at the three higher temperatures. Much larger samples were used than in 1925, and these were stored in large constant temperature rooms, in thin layers in shallow trays with slatted bottoms, to facilitate aeration and prevent heating. Samples were removed every 24 hours, shelled, and the pods and peas preserved separately. The methods of preserving and analyzing samples were the same as those previously used (**1**) in the developmental studies of the fruit.

Experimental results

Determinations were made for dry matter content, reducing sugars, sucrose, starch, acid-hydrolyzable polysaccharides, and crude fiber for both the 1925 and 1927 series.

COMPOSITION OF PODS AND PEAS

The results of the analyses of fruit stored at different temperatures are presented in tables I to VI. The initial composition of the pods or peas for the same component differed somewhat for the two years. This may have been due partly to environmental conditions, but was probably due mainly to the fact that there was a difference in the stage of maturity of the fruit at time of harvest. The higher percentage of dry matter and starch and the lower percentage of sucrose of the peas seem to show that the average fruit was slightly more mature in 1925 than in 1927.

DRY MATTER

As shown in table I, the *pods* of the fruit stored at 0–14° C. remained

TABLE I

PERCENTAGE OF DRY MATTER IN THE PODS AND PEAS

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	14.34	14.34	14.09	14.09	14.09
24	14.87	14.86	12.83	13.22	13.16
48	14.81	14.74	12.32	13.26	13.03
72	14.80	13.17	12.73	13.13	13.22
96	14.98	15.13	15.56	13.14	15.59
120	14.78	14.53	13.15	15.45	20.67
144	13.28	13.12	...		...
168		13.57	...		
	Peas				
0	23.01	23.01	21.69	21.69	21.69
24	23.02	23.37	22.02	22.62	23.25
48	23.67	24.66	22.39	24.48	23.55
72	23.44	24.34	23.29	24.54	24.02
96	23.42	25.58	23.49	25.07	25.26
120	23.40	25.96	24.19	26.62	27.14
144	23.18	25.96	...	...	...
168	23.63	25.67	...	...	...

rather constant in percentage of dry matter throughout the storage period. There was an increase at 25° C. and a somewhat greater increase at 35° C. This increase in the percentage of dry matter is probably due almost entirely to loss of water from the pods by transpiration. In the *peas* stored at 0° C. the percentage of dry matter remained almost constant, while at the higher temperatures it increased. For a time after harvest the water lost from the pods by transpiration is probably replaced by a withdrawal of water from the peas, causing the percentage of dry matter of the peas to increase before there is any noticeable change in the percentage of dry matter in the pods.

SUGARS

As shown in table II, the percentage of reducing sugars in the *pods* remained rather constant during the entire storage period at both 0 and 5° C., but at 14° C. there was a somewhat rapid decrease after 72 hours. At both 25 and 35° C. there was considerable loss in the percentage of reducing sugars, but the rate of loss was much more rapid at 35° C. The *peas* of the fruit showed a trace of reducing sugars after being stored 120 hours at 0 and 5° C., but there was no trace of them at the higher temperatures.

TABLE II

REDUCING SUGAR IN PODS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
0	13.69	13.69	12.15	12.15	12.15
24			12.25	11.38	9.80
48	12.31	13.11	12.06	11.85	9.60
72	12.72	13.99	12.42	9.32	8.45
96	13.64		10.60	8.31	4.30
120		12.71	10.01	5.53	1.66
144	14.18	12.68			
168		13.48			

Table III shows that there was a rather regular loss in the percentage of sucrose in the *pods* at all temperatures, the rate of loss increasing with the temperature. In the *peas* stored at 0° C. the percentage of sucrose remained almost constant for the entire storage period. At 5, 14, and 25° C. the rate of loss increased as the temperature was raised. At 25° C. about 34 per cent. of the sucrose was lost during the first 24 hours, and

TABLE III

SUCROSE IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	13.89	13.89	14.35	14.35	14.35
24	.	.	11.02	9.79	8.67
48	13.79		9.15	8.04	5.68
72	12.27	11.05	7.14	5.79	3.90
96	10.71	. .	6.70	4.01	1.90
120		9.63	5.62	2.30	1.24
144	9.51	7.61	..		. . .
168		7.88	..		
	Peas				
0	22.67	22.67	28.55	28.55	28.53
24	24.44	22.58	24.14	17.85	18.22
48	22.04	20.88	19.37	10.91	12.12
72	......	. ..	14.54	7.16	8.81
96	22.29	19.53	11.66	5.19	5.54
120	21.83	17.49	9.89	4.08	4.35
144	. ..	.	. .		
168	23.36	16.72	..		

after 5 days only about 14 per cent. of the original amount of sucrose remained. At 35° C. the rate of loss was slightly lower than at 25° C.

POLYSACCHARIDES

In the *pods* there was a reduction in the percentage of starch at all temperatures, the rate of loss increasing as the temperature was raised up to 25° C. (table IV). At 35° C. the rate of loss was about the same as at 25° C. In the peas stored at 0° C. there was very little change in the percentage of starch throughout the entire storage period. There seemed to be a slight increase up to 72 hours, then a slight decrease. In the peas stored at temperatures above 0° C., however, there was a definite increase in the percentage of starch. During the first 24 hours the rate of increase in the percentage of starch was most rapid at 35° C., but after the first 24 hours the rate of increase was most rapid at 25° C. The 35° C. temperature seemed to inhibit, to a certain extent, the formation of starch.

In the *pods* of the fruit stored at 0° C. the percentage of acid-hydrolyzable polysaccharides (starch deducted) did not seem to show any definite

TABLE IV

STARCH IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	6.63	6.63	7.98	7.98	7.98
24	7.11	6.57	5.59	4.37	5.25
72	5.94	6.13	4.30	2.74	2.52
120	5.51	5.47	2.85	1.59	1.83
144	5.10	...	...	...	...
168		3.93	...	...	...
	Peas				
0	16.06	16.06	12.36	12.36	12.36
24	16.27	17.23	14.15	17.56	18.93
48		...	...	22.00	21.22
72	17.51	18.22	18.73	24.21	22.66
120	16.97	18.22	20.58	23.40	21.85
144	15.38		...	...	...
168	15.68	20.06	...		...

TABLE V

ACID-HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED) IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
	Pods				
0	13.26	13.26	13.89	13.89	13.89
24	12.81	14.42	14.66	15.24	13.26
72	14.01	14.04	15.25	18.03	17.35
120	15.02	14.71	17.00	21.88	20.66
144	13.22	...		...	...
168		16.86	...		...
	Peas				
0	10.87	10.87	10.53	10.53	10.53
24	10.17	10.03	12.13	12.72	10.08
48	...	...	...	12.62	12.21
72	11.34	13.08	11.96	13.23	14.21
120	11.32	13.02	14.85	14.18	14.14
168	10.41	12.85	...	...	...

trend; at 5° C., however, there was a rather irregular but definite increase (table V). The most rapid increase in the percentage of acid-hydrolyzable polysaccharides in the pods occurred at 25° C. At 35° C. the rate of increase was less than 25° C. In the *peas* the percentage of acid-hydrolyzable polysaccharides remained rather constant at 0° C. At 5° C. there was a considerable increase during the first 72 hours of storage, but there was very little change thereafter. At 14, 25, and 35° C. there was a rather irregular increase in acid-hydrolyzable polysaccharides, and after 120 hours of storage there was very little difference in the amount that accumulated at these three temperatures.

TABLE VI

CRUDE FIBER IN PODS AND PEAS; EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	STORAGE TEMPERATURES				
	1925 SERIES		1927 SERIES		
	0° C.	5° C.	14° C.	25° C.	35° C.
			Pods		
0	14.96	14.96	14.30	14.30	14.30
24	14.77	15.29	15.75	16.52	16.86
72	15.68	15.94	11.76	19.24	20.79
120	16.34	16.37	18.62	23.87	25.97
144	15.82				
168		18.16			
			Peas		
0	8.37	8.37	8.41	8.41	8.41
24	8.29	8.41	10.07	10.85	10.19
48				11.32	10.61
72	8.38	8.55	10.86	11.28	11.35
120	8.41	8.62	11.17	10.93	11.05
168	8.46	8.55			

As shown in table VI, there was an increase in the percentage of crude fiber of the *pods* at all temperatures. The smallest increase occurred at 0° C. and the greatest at 35° C. In the *peas* held at 0 and 5° C. the percentage of crude fiber remained almost constant throughout the entire storage period. At 14, 25, and 35° C. there was a small initial increase, after which the percentage of crude fiber remained somewhat constant. There was very little difference in the percentage of crude fiber of the peas after 120 hours of storage at the three high temperatures.

TABLE VII

COMPOSITION OF PODS AND PEAS STORED SHELLED AND UNSHELLED AT 25° C., 1925 SERIES; COMPONENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

HOURS IN STORAGE	DRY MATTER IN PRESERVED MATERIAL		REDUCING SUGARS		SUCROSE		STARCH		ACID-HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)		CRUDE FIBER	
	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED	UNSHELLED	SHELLED
						Pods						
0	14.09	14.09	13.69	13.69	13.88	13.88	6.63	6.63	13.26	13.26	14.96	14.96
6		15.75		12.00		14.04						
12	13.66	15.01	12.55	12.41	12.27	14.20		6.28		13.50		15.14
24	13.74	17.49	12.42	12.01	11.07	11.53	6.69	4.28	13.36	13.01	16.39	15.81
						Peas						
0	23.50	23.50			22.67	22.67	16.06	16.06	10.87	10.87	8.36	8.36
6		23.41				19.89						
12	22.23	22.78			20.82	18.62		19.78		9.93		8.77
24	23.24	22.92			16.18	10.73	19.67	22.60	11.14	11.06	8.91	9.19

COMPARISON OF PODS AND PEAS STORED SHELLED AND UNSHELLED

In 1925 tests were made to compare the rate of change of some of the carbohydrate components in pods and peas when these were stored shelled and unshelled. The shelling was done immediately after harvest and the pods and peas were held at 25° C. Unshelled fruit was stored in the same chamber with the shelled. Samples were removed for analyses after 6, 12, and 24 hours.

As shown in table VII, the percentage of dry matter increased much more rapidly in the *pods* when they were stored shelled, probably because when the pods were broken the water had an opportunity to evaporate from the inner as well as the outer surface. The percentage of dry matter in the *peas* remained rather uniform throughout the 24 hours of storage. It is entirely likely that the high respiratory activity during this early period produced sufficient water to compensate for that lost by evaporation.

There was some loss in the percentage of reducing sugars and sucrose of the *pods* in both the unshelled and shelled series. In the *peas* the loss in percentage of sucrose was very rapid, a considerable drop having taken place even after six hours of storage. There was a marked difference in the rate of loss of sucrose between the two series, the percentage being reduced at a much more rapid rate in the shelled peas than in the unshelled. At the end of 24 hours about 53 per cent. of the sucrose had been lost in the shelled peas, while only about 29 per cent. had been lost in the unshelled. In the 1927 series (table III) the peas stored unshelled lost 37.5 per cent. of their sucrose during the first 24 hours of storage at 25° C. The initial percentage of sucrose, however, was considerably higher in the 1927 series, which may account for the more rapid loss. From this it would appear that the peas should not be shelled until ready for use, especially if they are to be held at ordinary room temperature. It would indicate also that the practice of shelling peas for market, as is sometimes done, is not a good one.

The data in table VII also indicate that a rapid decrease in the percentage of starch occurred during the 24 hours in the *pods* that were stored shelled. The percentage of starch in the pods stored unshelled, however, remained rather constant; but this does not agree with the data obtained in 1927, which show that the percentage of starch in the pods decreased from 7.98 to 4.37 during the first 24 hours (table IV). In the *peas* there was an increase in the percentage of starch in both the shelled and unshelled series, but the increase was much greater in those that were stored shelled.

There was very little change in the percentage of acid-hydrolyzable polysaccharides of the *pods* in either the shelled or unshelled series. In the 1927 series, however (table V), there was a definite increase in the percentage of these components during the first 24 hours. There was very little change in the acid-hydrolyzable polysaccharides of the *peas* during the first 24 hours. The small change that occurred seemed to indicate an accumulation, and is approximately the same for both the shelled and the unshelled series.

There was a slight increase in the percentage of crude fiber in the pods and peas of both the shelled and unshelled series.

Discussion

One of the most important aims of the grower and shipper of peas is to have the product reach the consumer in a condition of high edible quality. Providing harvesting is done at the proper time, probably the most important factor influencing quality is the temperature at which the product is held after picking. The preceding data show that in the carbohydrates studied there are no important changes taking place when the peas are stored at 0° C. In other words, at 0° C. these components remain in about the same proportion as they occurred at time of harvest. Cooking tests after a week at this temperature showed that the peas still retained their original fine flavor. BROWN (**2**), working with the variety Telephone, states that peas held for 40 days at 0° C. still had an excellent quality when cooked.

In the peas held at temperatures above 0° C. there is a loss in the percentage of the more mobile carbohydrates and an increase in the percentage of the more stable carbohydrates. These changes greatly affect edible quality, and appear to occur most rapidly at about 25° C. BROWN (**2**) states that when peas were stored at 10° C. they still had a good flavor on the fifth day, but declined to fair and poor on the seventh and ninth days; and when stored at 27° C. they were worthless after three days.

In most long-distance shipments of peas, ice is placed directly in the container. This keeps the material at approximately 0° C., and according to the preceding data, practically all of the important carbohydrate changes in the peas are held in abeyance while this temperature is maintained. In peas shipped long distances, therefore, the changes that affect quality unfavorably must occur before the peas are packed or after they are removed from the container. In order to place a product of high edible quality upon the table of the consumer, it is therefore necessary that the peas be put into the iced container as quickly as possible after harvest, and that they be held at a low temperature after they reach their destination at the terminal markets and in the homes of the consumer.

It is interesting to note that in the *pods* there is a decrease in starch while in the *peas* there is an increase in starch at the higher temperatures. The decrease in the percentage of starch in the pods can probably be accounted for to some extent by the increase in acid-hydrolyzable polysaccharides other than starch and by the increase in crude fiber. In the peas the increase in starch is probably due chiefly to the condensation of sugars. From the data at hand it is impossible to tell whether there is a movement of carbohydrates or other components from the pods to the peas after harvest.

Summary

PODS.—The percentage of *reducing sugars* in the pods remained somewhat constant at 0 and 5° C., but at the higher temperatures there was a gradual loss; the rate of loss was most rapid at 35° C. The percentage of *sucrose* decreased at all temperatures from 0 to 35° C.; the rate of loss was most rapid at 35° C. The percentage of *starch* decreased at all temperatures; the smallest loss occurred at 0° C. The losses at 25 and 35° C. paralleled each other rather closely. The percentage of *acid-hydrolyzable polysaccharides* changed but little at 0° C. The rate of increase was somewhat slower at 35 than at 25° C. The percentage of *crude fiber* increased at all temperatures. The rate of increase was least at 0 and highest at 35° C.

PEAS.—The percentage of *sucrose* in the peas remained somewhat constant at 0° C. during the entire storage period. The most rapid loss occurred at 25° C. The percentage of *starch* changed but little at 0° C., but there was a gradual increase at the higher temperatures. The decrease was most rapid at 25° C. The percentage of *acid-hydrolyzable polysaccharides* remained rather constant at 0° C. and increased but slightly at 5° C. At 14, 25, and 35° C. there was a rather irregular increase, but after 120 hours the percentage present was about the same in the three high temperature lots. The percentage of *crude fiber* remained about constant at 0 and 5° C. It also remained rather constant at the higher temperatures after a small initial increase.

FRUIT STORED SHELLED AND UNSHELLED.—In peas stored shelled and unshelled at 25° C., the most significant changes were the decrease in the percentage of sucrose and the increase in the percentage of starch. The sucrose decreased and the starch increased at a much more rapid rate in the shelled than in the unshelled peas.

The writers wish to express their appreciation to H. W. ALLINGER, of the Division of Chemistry, who made most of the analyses; also to J. G. SEWELL, of the Division of Chemistry, W. H. SPRAGUE, and MISS E. HARROLD, who helped prepare the material for analysis and did a portion of the analytical work.

BRANCH OF THE COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

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SEASONAL SHRINKAGE OF MONTEREY PINE AND REDWOOD TREES

FERDINAND W. HAASIS

(WITH FOUR FIGURES)

Introduction

It has for some time been a matter of observation that Monterey pine trees growing at Carmel, California, show a notable increase in diameter in the late fall or early winter months, apparently preceding the beginning of new spring growth. MACDOUGAL writes of one pine tree, for example, in which growth had ceased in August, as showing in late November and again in December "slight impulses of enlargement which are characteristic of the species in this region" (**10**, p. 10). MACDOUGAL (**7**, pp. 27–37; **8**, pp. 342–343) and LODEWICK (**5**), moreover, have reported slight shrinkages in the diameter of trees of several species at various seasons, but without attempting to correlate their observations very closely with external conditions.

Records of such phenomena have been obtained by means of the dendrograph, an instrument devised by MACDOUGAL in 1918 (**7**, pp. 10–15). This instrument may be described briefly as consisting of a rigid frame constructed of material with a low coefficient of expansion, suitably supported about a tree, and carrying two contact rods, one fixed and the other

FIG. 1. Dendrograph in position on a pine tree, showing floating frame, contact rods, lever assembly, recorder, and supports.

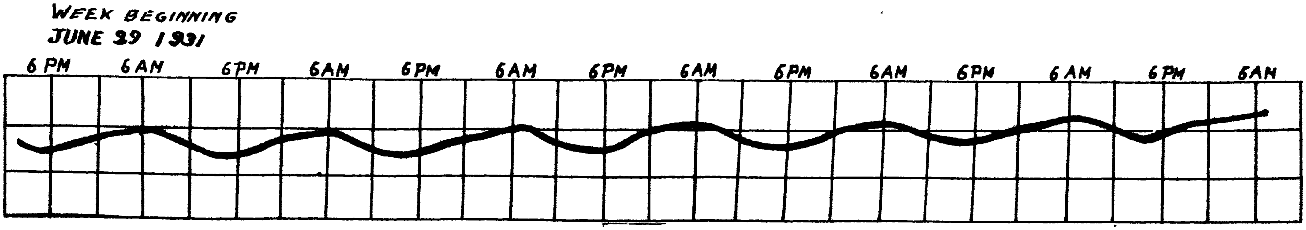

FIG. 2

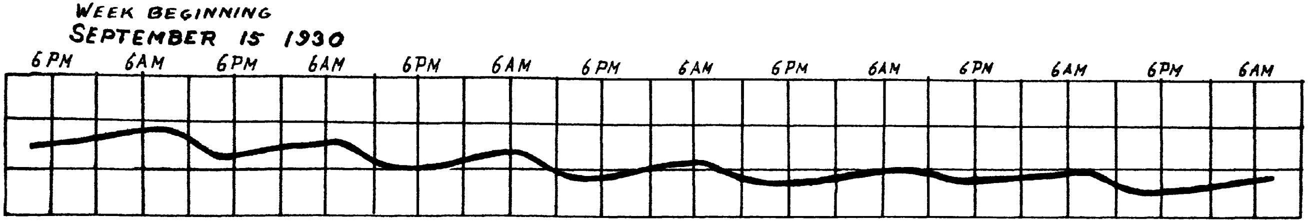

FIG. 3

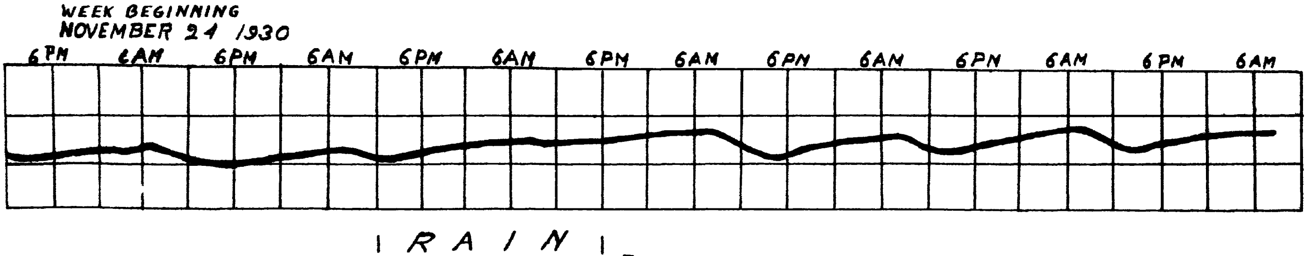

FIG. 4

FIG. 2. Reproduction of graph of a Monterey pine (no. 33) for the week beginning June 29, 1931, showing type of dendrographic record made by many kinds of trees in clear weather during growing season. Spaces between vertical lines represent 6-hour periods, between horizontal lines 10-mm. intervals on the sheet; ×11.

FIG. 3. Dendrographic record of a Monterey pine (no. 1–A) during period of progressive shrinkage in the fall of 1930. Daily reversible variations in diameter are shown, like those in figure 2, but the net change in diameter for the week is a decrease rather than an increase. Spaces between vertical lines represent 6-hour intervals, between horizontal lines 10-mm. intervals on the sheet; ×11.

FIG. 4. Dendrographic record of a Monterey pine (no. 1–A) showing reversal of diametral change in a week (November 24–December 1, 1930) when rain interrupted a period of dry weather. Rain began about noon of November 26 and continued through most of the following day, a total of 0.90 in. (23 mm.) falling; ×11.

movable. The movable rod actuates a pen lever, by means of which a continuous record of changes in diameter is made on a ruled sheet borne on a revolving cylinder. The general appearance of such an instrument is illustrated in figure 1. The direction of movement on the record sheet is at right angles to that of the movement between contact points.

The dendrographic records show that ordinarily, in clear weather, trees shrink during the day and swell at night. A week's graph, therefore, appears as a sinuous line. When growth is taking place, each successive nocturnal maximum is higher on the record sheet than the preceding one. A record of this type can be regarded as a normal graph for a growing tree. An example of this kind of graph is reproduced in figure 2.

Observations and discussion

SEASONAL VARIATIONS

The dendrographic records upon which this paper is based were made at Carmel, a short distance from Monterey, in Monterey County, California, and in the canyon of Rocky Creek, about 17 miles (27 km.) south of Carmel. Carmel is situated on a slight bay open to the Pacific Ocean. The trees under observation are about half a mile (1 km.) from the beach, located partly on top of an old fixed dune, partly on the northeast slope of this dune, and partly at its foot. The soil is markedly sandy. The trees at Rocky Creek are for the most part about a mile (1.5 km.) from the ocean, in a relatively narrow canyon. Here the soil is mainly residual, from granitic rock.[1]

In general, the climate of this region (which lies at about 36° N) is marked by a winter rainy season and a summer dry season. The latter can be expected to begin in May or June. It commonly lasts through August or September and may extend later, although not always absolutely without measurable precipitation for the entire period. The annual precipitation, on the basis of 21 years' records, varies from 9 to 27 inches (24–69 cm.), averaging 17 inches (43 cm.).

In the season of least precipitation, ocean fogs frequently cover the region, and as a result temperatures tend to be lower than in places farther inland. The combination of high atmospheric humidity and low temperatures greatly reduces the evaporating power of the air for much of the summer period. Frosts occur occasionally, both at Carmel and in Rocky Creek canyon, but for the most part the winters are relatively mild. On the whole the climate is comparatively equable, the extreme temperatures recorded in an instrument shelter on the fixed dune at Carmel ranging from 18° F. (−8° C.) in January, 1922, to 101° F. (38° C.) in October, 1917.

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[1] For detailed descriptions of the ecological features of this region see SHREVE (13, 14).

A detailed examination of the dendrographic records shows that an appreciable amount of shrinkage regularly occurs during the autumn prior to the fall or winter swelling which has been noted. This of course is especially marked in years when unusually long dry periods occur. Examples of such notably long dry periods in summer and autumn are afforded by the years 1926, 1929, and 1930. The records of several trees on the dune at Carmel show more or less continuous shrinkages during these essentially rainless periods. Typically these decreases in diameter begin in the latter part of the dry period; and with the advent of rain, diametral increases commonly occur.

These conditions are illustrated in figures 3 and 4. Figure 3 shows the kind of graph produced in September when a pine tree was shrinking. In figure 4 the effects are shown of a 0.9-inch rainfall occurring on the 26th and 27th of the month. It will be observed that the tree increased appreciably and rather steadily in diameter during this 2-day period, but again showed pronounced diurnal fluctuations on succeeding days.

Exceptions to this general program occur, which are apparently to be correlated with differences in soil moisture supply. For instance, one vigorous tree, situated in a swampy location at the base of the hill, did not begin to contract in 1929 until mid-November; whereas contraction in those on the dune in general had commenced at least a month earlier.

There also occur individual differences among the trees on the dune, one tree continuing to grow, for example, after others have begun to shrink. These divergencies can be ascribed partly to differences in root development of the several trees. In addition there appear to be pockets of moister soil here and there on the dune, where conditions for tree growth are more favorable than elsewhere. An irregular layer of hardpan formed of cemented sand is found at a depth of about 1 meter below the top of the soil. The upper surface of this hardpan is very irregular, and it seems likely that over a moderately extensive area there may be developed basins in which the moisture supply is considerably more abundant than at other, near-by, places. Trees which happen to start their growth over such pockets will be better able to continue growth during dry weather than those not so situated.

The records for the redwoods are less complete than are those for the pines, generally having been discontinued in the fall and resumed the following spring. Nevertheless the same general sort of seasonal shrinkage is shown. For example, in 1926 one tree, situated on a slope with southerly exposure, shrank appreciably in September and October. Another, on a north slope where moisture conditions were presumably more favorable, did not exhibit marked shrinkage until the latter part of October.

During the winter of 1930–1931, most of the dendrographs on redwoods were kept in operation throughout the season, just as were those on the pines. In this season, trees on both north and south slopes showed definite shrinkages in October, November, and December, followed by swelling early in January.

Evidently we are dealing with a drying out and a subsequent rehydration of the tree tissues. Directly after the beginning of a dry season, there will still be a certain amount of soil water available to the trees. Gradually, however, this supply becomes exhausted. In the summer or early autumn, the soil moisture content at a depth of 15–30 cm. may fall as low as 3 or 4 per cent. (on the dry weight basis) on the dune at Carmel. With the advent of rain, soil moisture again becomes available and the trees are enabled to make up the losses suffered during the dry period. It seems likely that the first diametral increases occurring after a dry period are not to be ascribed to the consequences of actual cell division, but simply to swelling of the partially dried tissues.

It should be pointed out that this seasonal shrinkage is not the same as absence of growth, but a decrease in diameter just as definite as the increases occurring during periods of cell division.

The essential identity of seasonal and diurnal variations in diameter will be shown in the succeeding section.

CONDITIONS AFFECTING SHRINKAGE AND REHYDRATION

Speaking of daily reversible variations, MacDougal has mentioned the fact that "contraction or shrinkage is an invariable attendant of lessened water-balance due to an excess of transpiration over water supplied" (9, p. 3). It seems obvious that seasonal shrinkage is to be explained in a similar way. Progressive shrinkage occurs when the soil is so dry that it cannot supply enough moisture to the tree to make up for the transpirational losses.

EVAPORATING POWER OF THE AIR.—It is to be supposed that when the evaporating power of the air is reduced, water is absorbed and a certain amount of swelling ensues. The assumption is justified by the fact that nightly diametral increases occur during a period of progressive shrinkage, the tree, however, suffering a net loss in diameter from night to night. If, therefore, the period during which the evaporating power of the air were low could be extended, it would seem that the progressive shrinkage could be checked or reversed. While it is not practicable to lengthen the period of darkness experimentally for a large tree, yet cloudy or foggy days do afford conditions under which the transpirational losses are somewhat reduced.

On one prevailingly cloudy day in May, several of the trees under observation shrank to a markedly less extent than they had been doing for some time past. Moreover, although shrinkage (or a static condition) had been evident for the past few nights, on this night definite increases occurred.

The question might be raised, whether the shrinkage and swelling which have been observed are due simply to drying of the thin layers of bark under the contact points of the dendrograph, and reabsorption of water from the moist air or from free water upon the bark itself. A number of observations and experiments have served to throw light on this question.

During a notably foggy night in April, several of the pines showed pronounced diametral increases as compared with the preceding night, although there had been progressive shrinkage for some time preceding. A conspicuous exception to this general performance was afforded by a small pine tree 1 cm. in diameter, in which shrinkage very definitely continued during this night. Presumably this is to be explained by considering that the roots of this small tree are not well enough developed to enable it to take in sufficient water for diametral increases, even when the evaporating power of the air is somewhat reduced. The contacts of the instrument on this tree are outside of the bark. The redwoods and the pines at the base of the dune, with ample water supplies, had been showing diametral increases prior to this foggy night, and the increased atmospheric humidity had no apparent effect upon their daily program.

It has been customary, in setting up the dendrographs on pines and redwoods, to seat the contact points on an area in which the bark has been thinned down to a thickness of about 2 mm. outside the cambium layer (MacDougal, 7, pp. 20–21). If this layer of bark were to swell appreciably when it became moist, the dendrographic records would obviously show an increase due to this cause; and duplicate instruments with contacts on different thicknesses of bark ought to show differences in amount of such increase. There was accordingly attached to a Monterey pine tree, carrying a dendrograph with the contacts on thin layers of bark, a second instrument with the contacts on the outside of the 1.5-cm. bark, which was operated for seven weeks during January and February. While the graphs from the two instruments are not absolutely identical, week for week, yet their agreement is remarkably close. If water is absorbed to an appreciable extent from the outside, then the instrument with contacts seated on the thicker bark would be expected to record greater fluctuations with alternating periods of moist and dry air than that with contacts on the thinner bark. On the other hand, if the intact bark were resistant to wetting while the exposed bark were not, differences in amount of fluctuation would still be expected. The conclusion accordingly emerges that swelling of the bark due to absorption of external moisture accounts for not more than a very small part of the diametral increase which is associated with rainy or foggy weather.

Further evidence along these lines is afforded by an experiment in which the bark in the vicinity of the contact points of a Monterey pine was wetted with tap water, no unusual increase in diameter resulting from this treatment. On the other hand, MacDougal reports experiments in which bast and cork tissue from Monterey pines was found to swell somewhat when placed in water (**7**, pp. 37–38).

Reduction of the evaporating power of the air can also be effected by lowering the temperature. This occurs at night and during stormy weather, in the latter case being especially associated with increased atmospheric humidity. Another way in which solar radiation can be cut off, with resulting temperature reduction, is exemplified by a partial solar eclipse around mid-day, which occurred in April, 1930. This was reflected in a reversal of the diurnal shrinkage among the pine trees. In the redwoods this swelling was less marked.

The intimate relation between shrinkage and evaporating power of the air is further brought out by the performance of the trees during extremely windy weather. Following a 10-day period with considerable rain and cloudy weather in February, 1931, there was a very clear day with a northerly wind at times reaching a velocity estimated at about 35 miles an hour. A general marked increase of diurnal shrinkage, both at Carmel and at Rocky Creek, was recorded on that day. On other windy days during the spring there has been a similar tendency toward exaggerated diurnal shrinkage. In the middle of April, 1931, the graphs of several trees at Carmel showed a maximum diameter about 8 A. M. for one prevailingly foggy day, while on the following day the greatest diameter was around 6 A. M. On this second day a pronounced southwest wind came up about 6 A. M.

On the other hand, when an easterly wind died down in the middle of the morning at Carmel, this fact was reflected by a decreased rate of diurnal contraction, or in some cases by an actual increase in diameter. Phillips reproduces two annotated dendrograms showing marked contraction, similar to that just noted, in hardwood trees in South Africa during periods when warm dry winds were blowing (**12**, pp. 46, 52, Pls. V, XI).

Soil moisture supply.—From what has already been said, it is obvious that the autumnal shrinkage is concurrent with a very dry condition of the soil, at least on the dune at Carmel. Judging by the low stage of Rocky Creek during the fall of 1930, it seems likely that in the case of the redwoods as well, an inadequate water supply is responsible for the observed shrinkage. Additional evidence of the correlation between soil moisture content and shrinkage is afforded by the performance of the trees in the spring of 1931. During a period of scanty rainfall at this time, shrinkage of the trees upon the dune was observed, while those at the foot of the dune were increasing in diameter. At the end of March the moisture contents of the soil on the dune and at the foot were, respectively, 8 per cent. and 27 per cent., at a depth of 30 cm. By the first of May the moisture in the former had dropped to about 5 per cent. The trees at Rocky Creek, also, exhibited growth during this period. While no soil sampling was done there, it seems reasonable, in view of the physical conditions in this canyon, to assume that these redwood trees were receiving a somewhat plentiful supply of moisture slowly seeping down from the higher elevations of the mountains.

In July, 1920, MacDougal performed an experiment in which water was supplied to a pine tree (**7**, p. 25). At the beginning of this month it was noted that the soil was very dry and that growth in this tree had essentially ceased. During a period of four days early in the month, 15,000 liters of water were applied to the soil near the base of the tree. Diametral increase began at once and continued steadily until August 16, the period being without rain. In this case it is obvious that increase in diameter is not attributable in the smallest degree to absorption of water by the bark, from the outside.

The facts just presented lead to the conclusion that there is a very close correlation between the moisture content of the soil and the seasonal shrinkage observed in Monterey pines and redwoods. The effects, of course, are modified by such atmospheric conditions as air moisture content, air movement, and air temperature.

When desiccation has proceeded so far that there is no longer soil moisture available for making up the transpirational losses, water is apparently drawn from the plant tissues themselves, and the result is the progressive shrinkage which has been observed. Evidently these Monterey pines and redwoods are able to withstand such desiccation without injury. This ability is in rather striking contrast to that of hardwoods accustomed to summer rainfall, which in a rare summer drought were observed to suffer marked leaf loss (Hursh and Haasis, **4**).

Pearson (**11**) recorded in 1924 that western yellow pine makes its greatest growth in both height and diameter during the driest month of the year. In this case, however, soil moisture seems adequate; and temperature is, perhaps, the controlling factor.

Other factors.—Brown (**2**, p. 237) has recorded observations of an early spring swelling as follows: "Growth in white pine [*Pinus strobus*] is divisible into (a) growth without cell division and (b) growth with cell division. The first begins as early as March [in the vicinity of Ithaca, New York] and the elements concerned (phloem) increase in radial diameter from 50 to over 100 per cent. The awakening of growth is due apparently to the rise of soil water with an accompanying increase in temperature.'' This writer speaks also of phloem contraction, after the end of September, which he attributes to extremely cold winter temperatures. BROWN'S studies were based on small blocks of phloem and xylem, 0.5 inch long and 0.25 inch wide, cut from the tree at frequent intervals (BROWN, **1**; LODEWICK, **6**, p. 14).

In view of what has just been said, it is well to question BROWN'S inclusive use of the term ''growth.'' It seems that his ''growth without cell division'' could more properly be referred to as ''rehydration,'' reserving the term ''growth'' for his ''growth with cell division.'' The extremely cold temperatures which he cites are not experienced in the Carmel region; and such conditions, therefore, cannot afford the explanation for the observed shrinkage here. The question, whether soil desiccation is as important as cold in New York, also cannot be answered without further study.

HALL (3), working at a latitude of about 35° S, noted that eight deciduous trees of four species showed a diametral decrease in May, apparently synchronous with the autumnal leaf fall. Eight evergreens, including one species of pine, did not show this decrease. HALL presents meteorological figures which show that May did not have the lowest mean temperature for the year, and that the precipitation was slightly above the monthly average, the 49-inch (124-cm.) rainfall being distributed rather uniformly throughout the year. In this case, of course, it is possible, as HALL suggests, that there was a definite relation between leaf fall and shrinkage. It can be assumed that the evergreens did not show the shrinkage because the soil moisture content did not become critically low.

Mr. A. D. LINDSAY, of the Australian Commonwealth Bureau of Forestry, has reported, in a conversation, somewhat similar shrinkages (or ''decrements'') in Monterey pine. These shrinkages appeared at certain seasons of the year in plantation trees, measurements being made monthly. There is no pronounced dry season in the region where these trees are growing, and the shrinkage cannot at present be definitely correlated with environmental conditions.

LOCALIZATION OF SHRINKAGE

In the case of a tree to which two dendrographs are attached, one 6.5 meters higher than the other, it is found that, in general, swelling or shrinkage takes place at about the same time at both heights. Occasionally, however, it happens that one of these instruments will show shrinkage or swelling while the other shows the reverse. The reason for these divergencies has not been ascertained.

Of perhaps even more interest is the performance of a small tree 1 meter in height. During the spring of 1931, the stem of this tree, at a height of 4 dm. above the ground, suffered a rather consistent shrinkage in diameter, a total of about 0.5 mm. during a period of nine weeks in March, April, and May. At the same time that this shrinkage was taking place, the terminal shoot continued to elongate, increasing in length a matter of 8 cm. or so.

From these observations it would seem that water may be drawn away from one part of a tree and used to keep turgid the cells in another part. The plant may be shrinking at one point and swelling at another. Apparently MacDougal's suggestion (9, p. 3) that the water deficit "would increase from the base of the trunk toward its summit" would not necessarily hold true under all conditions.

Another example of shrinkage in one part of a tree while another part is swelling is afforded by dendrographic studies of diametral fluctuations in different regions of the same individual tree. From such studies it appears that the heartwood of a Monterey pine may be shrinking while the tree as a whole is swelling.

Summary

1. Detailed analysis of dendrographic records indicates that the fall or winter swelling which has been observed by MacDougal in Monterey pine trees is preceded by a progressive contraction during the dry season of the summer and fall. It appears, furthermore, that such shrinkage may occur during an unusually dry spring. In either case, with the advent of rain—or sometimes in foggy weather—such changes in diameter may be checked or reversed. The fall or winter swelling is accordingly to be ascribed to rehydration of the desiccated stem, and probably is not connected with cell division. Redwood trees also are found to exhibit these seasonal variations, which are very closely related to MacDougal's previously described diurnal fluctuations.

2. Chief among the conditions affecting shrinkage and rehydration are the evaporating power of the air (dependent upon atmospheric humidity, temperature, and wind movement) and the amount of soil moisture available, the latter apparently being the more significant from the standpoint of seasonal fluctuations.

3. A tree may be shrinking in one part while it is swelling in another.

Carnegie Institution of Washington,
Carmel, California.

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CONTROL OF THE FLUX EQUILIBRIUM OF ELECTROCHEMICAL PROCESSES AND ELECTRIC POLARITY IN THE DOUGLAS FIR BY TEMPERATURE

E. J. LUND

(WITH TWO FIGURES)

Introduction

As an introduction to the following description of experiments on the effect of temperature on the electric polarity in the Douglas fir, it is necessary to distinguish between two distinct types of effects of temperature on the E.M.F. produced by an electrochemical system. These two types of effects depend upon whether or not the system is in thermodynamic equilibrium. The relatively simple relation between change in static equilibrium of an electrochemical system and temperature is defined by the GIBBS-HELMHOLTZ equation $E = T\frac{dE}{dT} - \frac{\Delta H}{nF}$, where E is the electromotive force, ΔH the change in heat content of the system, T the absolute temperature, n the number of electrochemical equivalents, and F the faraday.

The effect of temperature is in general relatively small and directly proportional to the absolute temperature. So far as is known, the same statement applies to the relation between temperature and a system in metastable equilibrium while it is in the metastable state. The essential feature of the metastable state is that a new state is assumed only after a certain amount of energy is added or withdrawn from the system. The "resting" or apparently "isoelectric" condition of any irritable system, such as a nerve or muscle fiber, resembles in this respect a non-living system in metastable equilibrium. If to this extent we view the phenomenon of excitation and response of a nerve or muscle as a change from a state of metastable equilibrium, without considering the unique feature of recovery of the living nerve or muscle, then we should expect to find that the effect of temperature on the P.D. at the surface (or injury P.D.) of a resting muscle cell would be small and proportional to the absolute temperature. This is just what was found, within limits of experimental error, by BERNSTEIN (1) in his original and important study of the injury potential in muscle. An instructive inorganic system is the passive iron wire model (LILLIE 2).

Any electrochemical system which at the moment of consideration expends electrical energy is obviously not in such a state of equilibrium. From this it follows that the continuous output of electric energy from polar cells and polar cell groups is derived from a system which never attains a condition of static, dynamic, or metastable[1] equilibrium in the sense of classical thermodynamics, but represents an output from a system in a constant state of flux of energy and material. I have designated the equilibrium state in such a system a *flux equilibrium* (LUND 6).

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[1] For definitions of different types of equilibria, see TAYLOR (9).

Since a system in a constant state of flux equilibrium depends for its existence upon the maintenance of both absolute and relative velocities of change in the linked processes of the system (for example, flame, whirlpool, or catenary series of chemical reactions), it follows that temperature may indirectly affect the instantaneous magnitude of the intensity factor of the energy change, for example, the E.M.F. of an electrochemical system in such a state of flux by affecting (1) the relative and (2) the absolute velocities of the individual links in the chain of reactions.[2]

In general we should expect that the temperature coefficient of the E.M.F. of such a system would be large (and often variable), provided that the effect of temperature alters the relative velocities of the individual members in the catenary series and therefore the E.M.F., since the flux concentrations of the electromotively active substances are changed. The variation of E.M.F. in the frog skin with temperature is apparently an illustration of these conditions (LUND and MOORMAN 3).

The following experiments on the Douglas fir furnish still more striking illustrations of the behavior of such electrochemical systems in living cells. In this connection it is interesting to recall the similarity between the phenomena of continuous and discontinuous output of electrical energy by living systems and, for example, the continuous output of light by bacteria and the discontinuous output of energy from the luminous organ of such a form as the firefly, in which the feature of apparent "metastability" has been added to the mechanism by the evolutionary process of specialization in function.

Effect of temperature on electric polarity of the apex

The simplest way to approach an analysis of the phenomena is by considering first the apexes of the main axis and lateral branches. Figure 1 shows a typical arrangement of the apparatus with the result shown in the curves below. In this experiment a lateral branch was cut from the tree and removed to the laboratory with a minimum of mechanical disturbance. The apex in its natural orientation with respect to gravity was inserted in the air jacket, which consisted of a round tin tube about 3.5 inches in diameter, soldered at each end to the edges of holes cut in the sides of the galvanized iron can. Two holes were provided with rubber stoppers (*h*) to permit access to the electrode contacts. The air chamber was lined with paraffined paper for electrical insulation. The electrode contacts were the

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[2] The change with temperature in relative velocities of the members of the catenary series would of course be defined by the difference in their temperature coefficients at the particular temperature under consideration.

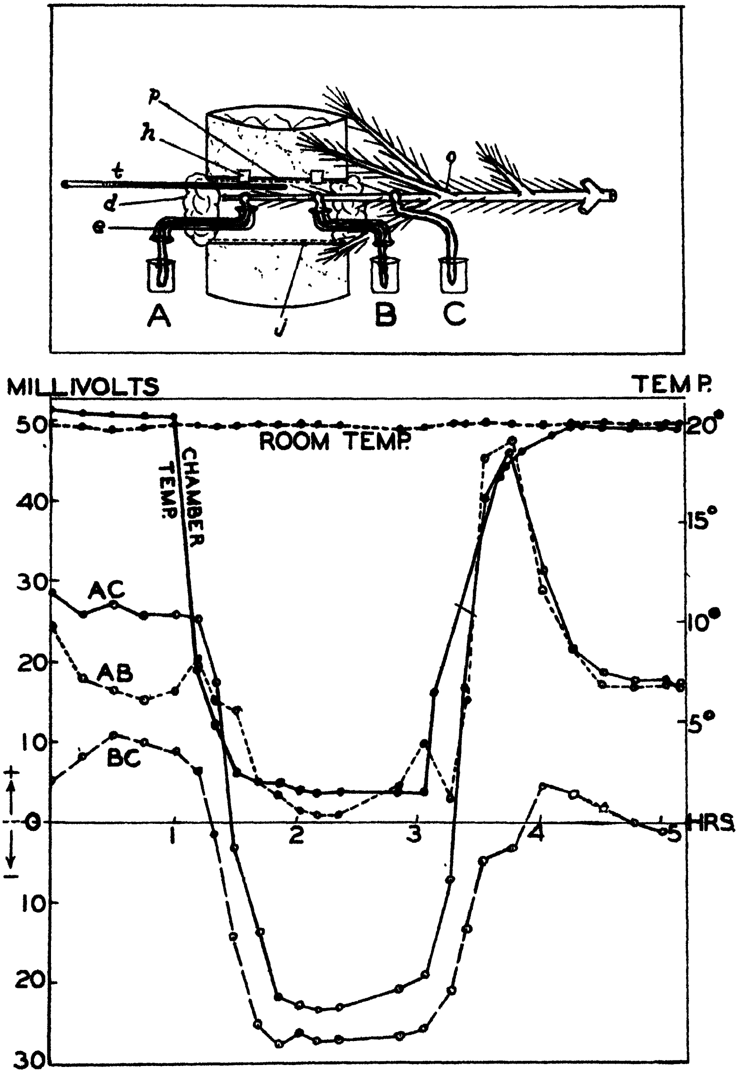

FIG. 1. Diagram at top shows sectional view of the arrangement. Open ends of the tin tube (*j*) are soldered to edges of holes cut in opposite walls of the tin vessel having a capacity of 3 gallons; heavy cotton insulation of vessel not shown. Stoppers (*h*) provide access to electrode contacts. Tin tube (*j*) is electrically insulated by paraffin paper lining (*p*); *t*, thermometer reading to 0.1° C. Dry cotton plugs (*d*) insulate the chamber. Glass tubes (*e*) contain the cotton contacts which dip into beakers with tap water, into which the Pb–$PbCl_2$ electrodes are dipped for contact. Curves *AB*, *AC*, and *BC* represent the change in magnitude and orientation of the electric polarity caused by temporarily cooling the segment *AB* from room temperature of 20 to 1.5° C.

usual cotton wicks kept saturated with tap water and inclosed in bent glass tubes (*e*). The cotton contacts dipped into a beaker filled with tap water. The electrodes, which are not shown, were hung for contact over the edge of the beakers during the measurement. Dry cotton plugs (*d*) were inserted in the ends of the chamber to prevent heat exchange. Air temperatures recorded on the graphs below were read off on a thermometer reading to 0.1° C., which was placed close to the apex. At the beginning of the experiments, tap water at room temperature was placed in the can, provided on the outside with heavy cotton insulation and an insulated cover not shown in the diagram. The electrode contacts were washed at intervals with tap water at room temperature and their P.D. checked before and after washing. As usual, insignificant variations of a millivolt or two occurred during renewal of the contacts. In most of the experiments it was found desirable to let the preparation stand at room temperature for about half an hour before readings of P.D. were begun, in order to permit effects of mechanical disturbance to disappear. The records of room and chamber temperatures are given by the curves in the figure.

Readings of P.D. between *A* and *B*, *A* and *C*, and *B* and *C* were taken systematically in the same order at intervals of from four to fifteen minutes. These values in millivolts are given in the curves. In this and following papers, the same convention of designating orientation of the polarity will be used as that employed previously (LUND 4). For example, when the P.D. between contacts *A* and *B* is given, as in curve *AB*, it means that contact *A* is electropositive to contact *B* in the external circuit. When the curve *AB* crosses the axis, the contact *A* becomes electronegative to the contact *B*, and so on. In other words, the first letter of the pair designating the curve indicates that the electrode designated by this letter in the figure is electropositive or electronegative to the electrode designated by the second letter, according to which point on the curve is considered.

In this experiment, readings of P.D. between *A* and *B*, *B* and *C*, and *A* and *C* were taken for one hour at constant room temperature. The water at room temperature was then drained from the container, the electrodes were washed through the holes with the stoppers (*h*), and the container quickly filled with a water-ice mixture. Readings of the air temperature in the chamber and P.D.'s were continued. At the end of three hours the water-ice mixture was replaced by water at room temperature, with a continuance of the readings. The distances between the contacts *A* and *B*, and *B* and *C* were respectively 13.5 cm. and 17 cm.

The extremely interesting results are simple and clear from inspection of the curves. They may be considered as follows. The P.D. between points *A* and *B* decreases to nearly zero value at about 1.5° C., and then increases to nearly 50 millivolts, returning to the normal value of about 16 millivolts at room temperature. Since the diameter of the apex between contacts *A* and *B* is practically the same and therefore cools at the same rate, the effect cannot be due to unequal rates of cooling of the two regions, especially when we consider the long time interval involved in the experiment. The only conclusion is that the P.D. in the more apical region *A* is decreased very much more than that in the less apical region *B* by the *same* lowering of temperature. That this interpretation is correct is shown by the curve *AC*, in which only the region of contact (*A*) is cooled, resulting in a decrease in P.D. to such an extent that it becomes electronegative to the region *C*, which is maintained at constant room temperature and therefore at approximately constant electric potential. Now when we compare the simultaneous change of P.D. in the region *B* with that in *C*, as shown by the curve *BC*, it is noted that the total change of P.D. under the same conditions is less in region *B* than that in region *A*.

The second remarkable difference between the effect of temperature on the apical region *A* and that of the more basal region *B* is shown by the large "rebound" of the curves *AB* and *AC* as compared with the small "rebound" of the P.D. in region *B* in curve *BC*. It is clear that the same lowering of temperature around the apex tends to diminish the normal electric polarity; and if the lowering of temperature is sufficiently great, the electric polarity disappears and finally becomes inverted. Return to room temperature results in a return to the normal orientation of the polarity, which is followed by a great temporary increase in P.D. and then a return to normal. Experiments on the tip showed that the effects of temperature are definitely graded in their magnitude from the tip downward. This fact is further illustrated in the following experiment.

Effect of temperature on electrical polarity in a basal segment of apical internode

The diagram at the top of figure 2 shows a typical experimental arrangement when the apex of a lateral branch from near the top of a 6-year old tree was used. Contacts *D* and *C* are inside the chamber which surrounds the segment to be subjected to change in temperature. The distances between the pairs of electrode contacts *A* and *B*, *B* and *C*, *C* and *D*, *D* and *E*, and *E* and *F* are respectively 11 cm., 13 cm., 13 cm., 19 cm., and 11.5 cm. The results are shown below in the graphs, all of which are drawn to the same scale but separated into two groups for the sake of avoiding confusion in making comparisons. The curve of segment *AB*, not subjected to change in temperature, shows only a slight effect if any of temperature on its polarity by the change in the distant segment *DC*. Segment *AB* has a normal average electric polarity of about 18 millivolts. The total duration of the experiment was eight hours.

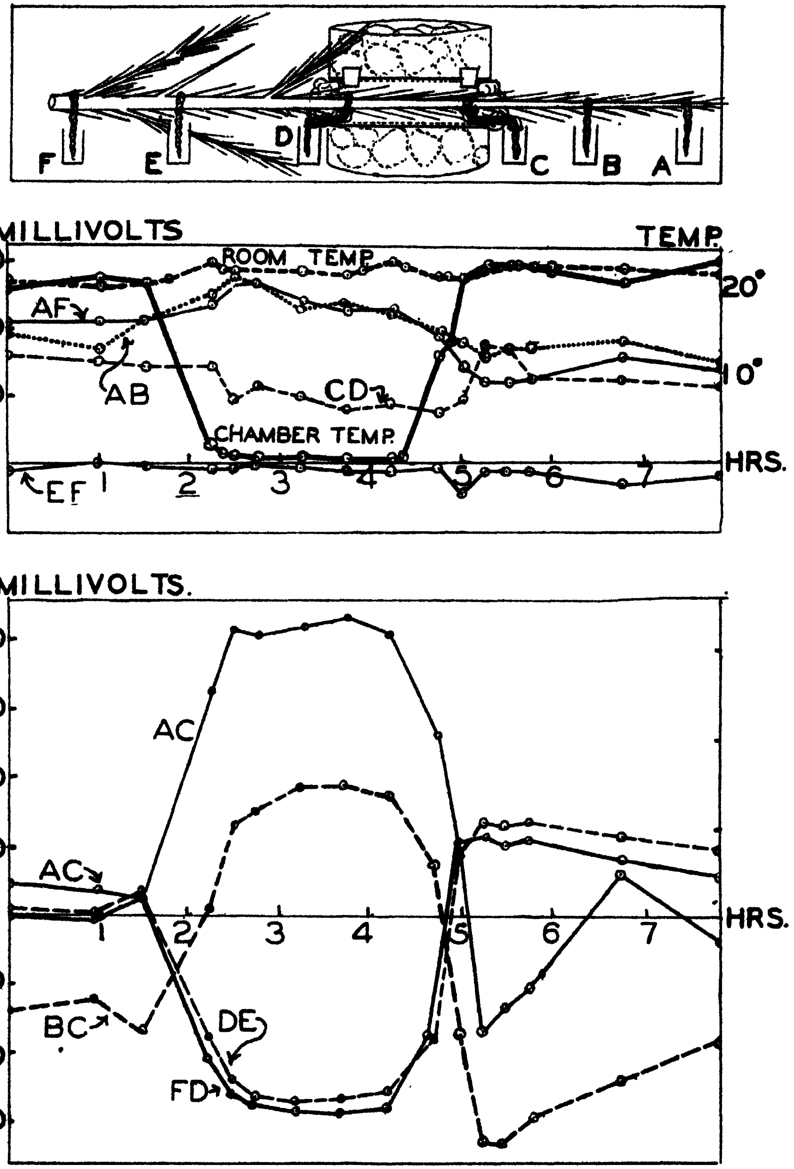

FIG. 2. Diagram at top indicates the arrangement, details of which are given in figure 1. Curves indicating the drift in E.M.F.'s of the correspondingly lettered segments are separated into two groups to facilitate comparison. The two sets of curves are drawn to same scale and may be superposed. Comparison should be made of each curve with the corresponding ones in figure 1. Lengths of the segments *AB*, *BC*, *CD*, *DE*, and *EF* are respectively 11 cm., 13 cm., 13 cm., 19 cm., and 11.5 cm.

At room temperature the polarity of the segment *BC* was *inverted.* This condition usually never occurs except after mechanical stimulation. When the temperature around *C* is lowered, its P.D. is lowered to such an extent that the electric polarity becomes oriented in the normal direction. Return to room temperature results in increase in the electropositivity of the region *C,* with the temporary "rebound" of E.M.F. This is another illustration of the important general fact that the "rebound" is not as marked in lower levels of the stem as it is in the apex (*cf.* figs. 1 and 2).

At room temperature *A* is slightly electropositive to *C;* but lowering the temperature lowers the P.D. in the region *C,* with the result that the segment *AC* attains a large polarity followed by a marked "rebound" of the E.M.F. in the region *C* which temporarily *inverts* the polarity of *AC.* The existence of the "rebound" is unmistakable but smaller than that in the apex as shown in figure 1.

At room temperature *DE* has little or no polarity, but cooling causes the appearance of an inverted polarity with return to a normal polarity associated with a smaller but distinct "rebound" which tends to persist. The change in segment *FD* follows closely the change in *DE.* This merely shows that it is the E.M.F. in the region *D* which is affected and not that in regions *E* or *F.*

In the upper set of curves, *CD* shows that the P.D. between the regions *D* and *C* becomes distinctly less at low temperature. But the difference in the effect of temperature upon the apical region *C* and the more basal region *D* is relatively small in comparison with the difference in a more apical region, such as in figure 1, curve *AB.* The evidence in the curves for a variation of E.M.F.'s in the segments *AB* and *EF* is apparently indefinite and needs further detailed investigation.

The question whether or not lowering of the temperature of the segment *DC* lowers the E.M.F. between *F* and *A* is of special interest when considering the validity of the principle of summation of E.M.F.'s in polar tissues. Since the drop in E.M.F. in *CD* is relatively small, the relatively small change in the curve *AF* may have some significance. From experiments carried out in a different manner, it appears that the procedure as indicated in figure 2 is not well adapted to bring out the facts on this point, especially when changes in temperature on segments from the more basal levels of the stem are observed. This question will be touched upon again in the following paper. (See also MARSH **8**.)

Significance of results

From the facts presented in previous papers (LUND **4**, **5**), it appears clear that the E.M.F.'s which constitute the characteristic polar properties of the Douglas fir have their origin in the living cells. What is observed when measuring the external longitudinal electric polarity, as in these experiments, is the resultant of a rather complex system of E.M.F.'s with two main groups of structural origins, namely, living wood and living cortex, both of which are derived from the bipolar cambium. It has also been fully shown that the fundamental electrical characteristics of a polar group of cells are directly referable to the same characteristics in a single cell. The facts are summed up in the statement that the electric polarity of the organ is the algebraic sum of the electric polarities of its cells.

Now the only way, from the view-point of electrochemistry, in which it is at present possible to think of the electric polarity of a cell, is that this E.M.F. is the algebraic sum of elementary regional or phase boundary (loci) potentials having an aggregate polar orientation. The simplest expression which defines the conditions of such a system is that for two oppositely oriented potential differences.

If for the moment we assume that this system is an oxidation reduction system in the state of flux equilibrium, and for the sake of simplicity consider only two of the electromotively active species of reactants in the two loci in the cell, then for the polarity potential of the cell we may write

$$E_p = \frac{RT}{2F} \ln \left(\frac{[A]_b\ [AH_2]_a}{[AH_2]_b\ [A]_a} \right), \qquad (1).$$

In order to make this expression apply to a large number of loci, we should in the ideal case merely have to sum all the elementary E.M.F.'s in a complex system in order to obtain an expression representing the total E.M.F. (*cf.* LUND **6**, p. 245).

With this conception in mind, the important question is, how would temperature be expected to affect the E.M.F. of such a polar system? If we assume for the moment that the above equation represents the E.M.F. of a system in *static* equilibrium, such as a simple concentration potential, then it is evident that the E.M.F. would be directly proportional to the absolute temperature, for the ratio of concentrations of A and AH_2 in any of the loci would not be changed in such a state of perfect reversibility. The observed effects of temperature on such a system are of course relatively small and similar to what BERNSTEIN found for the resting and injury potential (*cf.* metastable state, above) in a muscle.

Now if we consider the total E.M.F. of two oppositely oriented homologous cells, one located in the apical region *A* and the other in the more basal region *B* in figure 1, then the expression for the total E.M.F. becomes

$$\Sigma E_p = \frac{RT}{2F} \left[\ln \left(\frac{[A]_b\ [AH_2]_a}{[AH_2]_b\ [A]_a} \right)_1 - \ln \left(\frac{[A]_b\ [AH_2]_a}{[AH_2]_b\ [A]_a} \right) \right], \qquad (2).$$

The subscripts 1 and 2 denote respectively apical and basal cells. Again it is obvious that the change in total E.M.F. with *equal* change in temperature around the apical and basal cells is small and directly proportional to the absolute temperature. Lowering of the temperature of such a system obviously could not produce as great an effect as that represented by the drop in curve *AB* in figure 1. It could not at all account for the "rebound" phenomenon shown by curve *AB* when the system is returned to room temperature.

These facts, together with the further fact that the system is an open one which generates energy, dispose completely of all attempts to view the equilibria of these *continuous* bioelectric currents associated with life activities in terms of simple concentration potentials of systems in static, metastable, or dynamic equilibrium.

The only obvious alternative way in which these effects of temperature on electric polarity can be explained is that change in temperature changes in a characteristic manner the relative flux concentrations of the corresponding electromotively active substances in the apical and basal regions *A* and *B*. Since the electrochemical system is in a state of flux equilibrium, the same change in temperature must affect *unequally* the velocities of the individual links in the catenary series of reactions which are in the state of flux equilibrium.

In short, here is an indirect method of showing that the temperature coefficients of the velocities of the different links in the chain of reactions which constitute the mechanism for producing electrical energy by the cell are different. Raising or lowering the temperature results in increasing or decreasing selectively the concentration of certain electromotively active components at the seat of origin of E.M.F., thus changing the ratio of the concentrations in equation (2) above. When the system is returned to the former temperature, then the concentrations of the links in the catenary system return approximately to their former relative values. This is the explanation of the "rebound" phenomena in the curves. The magnitude of the "rebound" is determined by the magnitude of the unequal changes of the concentrations. These facts, especially when taken in conjunction with (a) the "rebound" effects on the E.M.F. in the frog's skin caused by changing the oxygen concentration; (b) the "rebound" effects produced in the output of CO_2 by *Planaria* after a period in the absence of oxygen; and (c) the unequal effects of equal change in oxygen concentration on the velocity of oxidation in apical and basal ends of the *Obelia* stem (LUND **3**, **7**, **6**), constitute a conclusive proof that the electrochemical mechanism which produces continuous bioelectric currents in the living cell is in a state of flux equilibrium.

The question remains, is this system in the Douglas fir an oxidation reduction system which is linked quantitatively with cell oxidation as has been shown in the case of *Obelia*, roots, and the frog's skin? In the absence of experimental facts an answer to this question is postponed.

Another interesting question is, how are the directions and magnitudes (patterns) of the internal correlation currents in the wood and cortex modified when the polarities are changed by temperature? A clearer understanding of how change in temperature can be used to modify this pattern of distribution of correlation potentials is of obvious importance in the practical solution of the problem of how such correlation processes as inhibition and dominance of growing points operate in the developing organism. In a following paper further light will be thrown on some of these questions.

Summary

1. Additional evidence is presented to show that temperature affects the E.M.F. of a system in flux equilibrium by changing the relative concentrations of the electromotively active substances which represent the links in the catenary series of reactions in flux equilibrium. The other effect of temperature on such a system is small and proportional to the absolute temperature, but is purely imaginary since the system is not reversible and therefore is not in thermodynamic equilibrium.

2. By lowering the temperature equally around an apical segment of the main axis or lateral branch of the Douglas fir, the electric polarity of this segment is diminished. This is due to unequal effect of equal change in temperature on apical and basal regions of the piece, and is related to the difference in the amount of change in the ratios of concentrations of the electromotively active substances in apical and basal levels of the piece.

3. Return of the previously cooled segment to the original temperature results temporarily in what has been called a rebound of the E.M.F. This rebound is relatively greater in apical than in basal regions of the stem. This phenomenon is a logical consequence of the nature of the effect of temperature on the flux equilibria.

4. The electric polarity of the apex of the Douglas fir may be increased, decreased, reversed, or made equal to zero by means of temperature. By inference, the internal correlation currents are therefore changed in a corresponding manner. The possible significance of these phenomena for regulation in growth and other processes is pointed out.

THE UNIVERSITY OF TEXAS,
AUSTIN, TEXAS.

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FLOWER PRODUCTION FROM GLADIOLUS CORMS HARVESTED AT DIFFERENT STAGES OF RIPENING[1]

B. E. Gilbert and F. R. Pember

Differences of opinion have been found to exist among commercial gladiolus growers as to the proper stage of maturity at which to harvest gladiolus corms to insure later maximum flower production. Some growers are very careful to allow the tops to dry out before digging the corms; others dig immediately after flower production is past and allow the plants to dry out exposed to the sun and air before cutting off the tops; while others claim that neither of these precautions is necessary and that as good flower production may be secured by digging immediately after the flowering period and severing the green tops from the corms at once.

An experiment was carried on during 1930 and 1931 at the Rhode Island Agricultural Experiment Station to determine the effect on yield of blossoms of plants grown from corms harvested according to these methods.

Cultural methods

During the spring months of 1930, plants of the Alice Tiplady and Maiden's Blush varieties were grown in the greenhouse in fertile soil to which optimum nutrients were added. Both varieties flowered during the months of May and June. The Alice Tiplady plants were harvested on three different dates, with the following treatments:

1. July 14: 25 plants harvested and taken for chemical analysis.
   20 plants harvested and topped at once.
   20 plants harvested and allowed to dry in the sun with the corms covered, and topped September 11.
2. August 7: 25 plants harvested and taken for chemical analysis.
   20 plants harvested and topped at once.
   20 plants harvested and allowed to dry in the sun with the corms covered, and topped September 11.
3. September 11: 100 plants dried out in the bed, harvested, and topped.
   25 plants taken for chemical analysis.

Two harvests were made with the Maiden's Blush plants, and treatments were as follows:

1. July 17: 400 plants were harvested and of these
   100 were topped at once,
   25 were taken for chemical analysis,
   275 were stored exposed to sun and air with corms covered.

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[1] Contribution no. 417 of the Rhode Island Agricultural Experiment Station.

2. August 7: 25 of the preceding 275 taken for chemical analysis.
3. September 11: Remainder topped.

The corms which had been harvested as indicated were stored in a cool dry cellar throughout the summer and were planted in the greenhouse on February 19, 1931, in fertile soil to which optimum nutrients had been added. The plants from these corms bloomed during May and June, thus giving data showing the effect of harvesting methods on growth and yield.

Methods of chemical analysis

Essentially the same procedure as that used by POTTER and PHILLIPS (**1**) with apple spurs was followed in preserving, extracting, and fractionating, with the following exceptions:

PRESERVING THE SAMPLE.—Immediately after harvesting, 25 plants were brought to the laboratory. The corms were severed from the tops at the base of the stalk. The tops and corms were quartered and one-quarter of each was cut up into fine pieces and then ground through a Nixtamal mill. The ground material was weighed. Two samples of 10 gm. each were weighed into tared moisture dishes and the moisture determined. Two samples of 100 gm. each were weighed into tared Erlenmeyer flasks, 0.25 gm. $CaCO_3$ and 150 cc. hot 95 per cent. alcohol added, and the flask heated to gentle boiling for 30 minutes.

DETERMINATION OF CARBOHYDRATE FRACTIONS.—Sugars were determined by the use of the SHAFFER-HARTMAN iodimetric method (**2**) for the estimation of cuprous oxide.

A major exception from the methods of POTTER and PHILLIPS was found to be necessary in the manner of estimating acid-hydrolyzable polysaccharides. It was found impossible to secure consistent results from the acid hydrolysis of the residue from the starch determinations, and so the acid-hydrolyzable polysaccharides were determined on 1 gm. of the dried residue. Thus this fraction in this experiment contains starch and is essentially different from that of POTTER and PHILLIPS.

Yield data

In table I data are given showing the yield of blooms and corms from plants grown from corms which had been harvested in various ways.

Alice Tiplady plants were harvested on July 14, August 7, and September 11, 1930, and immediately topped. Plants grown from these corms in 1931 gave no indications of significant differences in yields of corms or flower stalks, considering a 20 per cent. difference to be the lower limit of a significant yield variation. There was, however, a consistent increase in the average length of flower stalk and number of florets as the 1930 corms were allowed to remain longer in the bed.

TABLE I

YIELD OF GLADIOLUS PLANTS GROWN FROM CORMS MATURED BY DIFFERENT METHODS

VARIETY	HARVEST 1930	TOPPED 1930	BLOOMS, 1931			CORMS, 1931	
			No.*	AVERAGE LENGTH	AVERAGE NO. PER PLANT	No.*	AVERAGE WEIGHT PER CORM
				inches			*gm.*
Alice Tiplady	July 14	July 14	100	28.5	8.0	105	12.8
	Aug. 7	Aug. 7	100	29.0	8.5	95	20.6
	Sept. 11	Sept. 11	111	32.0	9.0	105	16.9
	July 14	Nov. 11	115	28.0	8.0	130	16.1
	Aug. 7	Nov. 11	95	26.5	7.5	110	17.5
Maiden's Blush	July 17	July 17	101	32.5	11.5	100	25.0
	July 17	Nov. 11	102	33.5	11.0	112	25.8

* Based on 100 plants.

Corms from Alice Tiplady plants harvested July 14 and August 7, 1930, and allowed to dry out with the tops exposed to the sun and air, produced plants in 1931 which yielded essentially the same in blooms and corms. There were no appreciable differences in the average lengths of flower stalks or average number of florets.

The Maiden's Blush corms which were harvested July 17, 1930, and immediately topped, when compared with similar corms harvested on the same date and the tops allowed to dry in the sun, gave plants in 1931 which were essentially the same in all yield data.

Chemical determinations

The results of the determinations of the carbohydrate and nitrogen fractions are given in tables II to V. Attention is drawn particularly to tables III and V, where the results are expressed as actual grams of the analytical fractions in 25 plants of the two varieties of gladiolus.

As noted previously, the Alice Tiplady plants (table III) which were analyzed were harvested July 15, August 3, and September 11, 1930. The plants harvested September 11 might be considered to have been metabolizing actively until the middle of August at the least. After that time the tops gradually dried out, until when harvested they were air-dry. Thus the acid-hydrolyzable polysaccharides and the alcohol-insoluble nitrogen accumulated in the tops and corms. Apparently the greatest increase must have been in cellulose, although this cannot be demonstrated as that fraction

TABLE II

Analytical fractions expressed as percentages of dry matter (Alice Tiplady)

Date of harvest	Tops			Corms		
	July 15	Aug. 3	Sept. 11	July 15	Aug. 3	Sept. 11
Dry matter*	20.96	24.58	93.10	24.67	29.65	31.65
Reducing sugar	5.30	4.13	0.74	2.89	1.79	0.82
Sucrose	Trace	1.38	Trace	7.42	7.47	4.49
Starch	Trace	Trace	Trace	46.17	47.83	47.39
Acid-hydrolyzable polysaccharides	1.48	1.57	0.96	37.19	40.24	38.55
Alcohol-soluble nitrogen	0.27	0.15	Trace	0.71	0.63	0.81
Alcohol-insoluble nitrogen	1.28	0.95	0.74	1.15	1.34	1.85
Total nitrogen	1.55	1.10	0.74	1.86	1.97	2.66

* In the undried material.

TABLE III

Actual grams of analytical fractions in 25 plants (Alice Tiplady)

Date of harvest	Corms			Tops		
	July 15	Aug. 3	Sept. 11	July 15	Aug. 3	Sept. 11
Dry matter	209.60	444.06	837.90	394.72	405.61	443.10
Reducing sugars	11.12	18.27	6.21	11.41	7.27	3.66
Sucrose	Trace	6.09	Trace	29.31	30.30	19.89
Starch	Trace	Trace	Trace	182.24	194.00	209.98
Acid-hydrolyzable polysaccharides	3.10	6.97	8.04	146.80	163.22	170.81
Alcohol-soluble nitrogen	0.56	0.67	Trace	2.79	2.56	3.59
Alcohol-insoluble nitrogen	2.68	4.22	6.20	4.54	5.43	8.20
Total nitrogen	3.24	4.89	6.20	7.33	7.99	11.79

was not included in the analysis. In the corms the starch fraction increased with maturity, partly at the expense of the soluble sugars, which decreased. The total nitrogen of the corms may have increased owing to the translocation of soluble nitrogen from the tops. In both carbohydrate and nitrogen fraction the movement was toward the accumulation of storage forms in the corms. This is as would be expected with storage in corm formation.

Table V gives the results of the analysis of two lots of 25 Maiden's Blush plants which differed only in the treatment after harvesting. The tops of the plants which were allowed to dry out in the sun apparently were sub-

TABLE IV

ANALYTICAL FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER (MAIDEN'S BLUSH)

DATE OF HARVEST	TOPS		CORMS	
	JULY 17	AUG. 3	JULY 17	AUG. 3
Dry matter*	26.39	89.70	30.52	33.70
Reducing sugar	5.49	0.15	1.03	0.69
Sucrose	0.61	Trace	5.86	4.90
Starch	Trace	Trace	53.62	44.23
Acid-hydrolyzable polysaccharides	2.15	2.08	35.52	41.52
Alcohol-soluble nitrogen	0.18	0.26	0.56	0.58
Alcohol-insoluble nitrogen	0.62	0.50	2.10	1.13
Total nitrogen	0.80	0.76	2.66	1.71

* In the undried material.

TABLE V

ACTUAL GRAMS OF ANALYTICAL FRACTIONS IN 25 PLANTS (MAIDEN'S BLUSH)

DATE OF HARVEST	TOPS		CORMS	
	JULY 17	AUG. 7	JULY 17	AUG. 7
Dry matter	263.90	259.00	488.32	539.20
Reducing sugars	14.50	4.59	5.02	3.71
Sucrose	1.60	Trace	28.60	26.45
Starch	Trace	Trace	261.84	238.49
Acid-hydrolyzable polysaccharides	5.67	0.54	173.45	223.87
Alcohol-soluble nitrogen	0.49	0.79	2.73	3.15
Alcohol-insoluble nitrogen	1.64	1.29	10.25	6.09
Total nitrogen	2.13	2.08	12.98	9.24

ject to translocation from tops to corms, as all the fractions (with the exception of the soluble nitrogen) decreased materially in actual amounts, while there was an increase in the acid-hydrolyzable polysaccharides in the corms. It is suggested that while the decrease in the reducing sugars and sucrose in the corms may have been due partly to respiration, the greater part of the loss in these fractions was due to condensation to polysaccharides. The total nitrogen of the corms decreased, owing to a marked decrease in the insoluble nitrogen.

Discussion

In general, the changes noted by the analysis of carbohydrate and nitrogen fractions are in line with the theory of condensation of simple compounds to complex storage forms which normally takes place during ripening. Theoretically, therefore, the additional amounts of condensed compounds in the corms which had been allowed to mature before being dug, or having the tops severed, might be expected to give the better plants and the better yield. Referring to table I, however, there seems to have been but slight differences between the yields of the 1931 crop despite the harvesting treatment of the 1930 plants. No appreciable size differences were noted in connection with the Alice Tiplady corms harvested at different dates. This fact is also in agreement with the data in table I. From other unpublished data it has been determined that size of corm affects flower production. Where such differences have been noted, however, the size differences have been many times greater than any noted in this experiment. This leads to the conclusion that differences such as were found to be present in amounts of storage chemical compounds, were perhaps not great enough to influence the succeeding generation of gladiolus. In other words, usually a gladiolus corm may be considered to have huge reserves of energy, only a portion of which is needed to establish and maintain the new plant.

Conclusions

From data obtained by growing gladiolus in the greenhouse from corms, some of which were harvested at different stages of maturity, some of which had the tops removed immediately after harvest, and some of which were allowed to dry out before topping, it is concluded that there is little to gain by any one of these procedures over the others. Differences in flower and corm yields were not significant. There seemed to be a slight benefit in length of flower stalk and number of florets accompanying greater maturity; for example, the Alice Tiplady corms which were allowed to mature in soil gave longer stalks and more florets per stalk.

Chemical analyses gave data showing accumulation of condensed forms of carbohydrates and nitrogen compounds stored particularly in the corms. These additional amounts, however, were apparently not great enough to influence the yield of the succeeding generation of gladiolus.

RHODE ISLAND AGRICULTURAL EXPERIMENT STATION,
KINGSTON, R. I.

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FIELD METHOD FOR ESTIMATING NITRATE, PHOSPHATE, AND POTASSIUM IN PLANTS[1]

E. M. Emmert

Introduction

Work on tomato and lettuce plants (**2**) indicates that the percentage of a plant-food element in the mature conducting tissue of a plant is a better index of the adequacy of the soil in supplying this element to the plant than is the percentage of the element found in the soil. Therefore quick tests for approximating the percentages of nitrate, phosphate, and potassium in mature plant tissue, in the field, should have practical value in supplying information to guide the application of fertilizers, either to the growing crop or for the benefit of a crop to follow. The procedures described in this paper, which are adaptations of good laboratory procedures, seem to be satisfactory for the purpose indicated.

The field pack should be as follows:

For preparing the plant extract.—A small hand balance and 5 gm. weight; a 10 cc. graduate; two small mortars and pestles; 11 cm. no. 1 Whatman filter-papers; test-tubes (3½″ × ½″ is a convenient size); a test-tube rack; a dipper to measure 0.5 gm. of charcoal; a 1 cc. pipette graduated in tenths of a cc.; glacial acetic acid; phosphate-free charcoal (**3**); tap water (distilled water is best if available). Besides these, calcium hydroxide and a 10 per cent. solution of copper sulphate (**4**) may be needed for some tissues.

For the nitrate test.—A 25 cc. evaporating dish; an alcohol lamp; a pair of tongs; a 25 cc. graduate; test-tubes (3½″ × ½″); phenoldisulphonic acid (**1**) (solution An); clear 30 per cent. sodium hydroxide (solution Bn); a set of color standards, made as directed under the nitrate test.

For the phosphate test.—A 1 cc. pipette; a 2.5 per cent. solution of ammonium molybdate in 5 N sulphuric acid (solution Ap); aminonaphthol sulphonic acid (solution Bp) (**6**); a set of color standards, made as directed under the phosphate test.

For the potassium test.—A 25 cc. evaporating dish; sodium cobaltinitrite solution (**5**) (solution Ak); a solution made by dissolving 27 gm. of sodium nitrite in distilled water and making up to 100 cc. (solution Bk); a set of color standards, made as directed under the potassium test.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Preparation of plant extract

Portions of the lower mature main stem or of lower mature petioles or branches as near the main stem as possible should be used. Immediately weigh 5 gm. of the fresh tissue as nearly whole as possible and cut into small pieces after weighing. Grind in a mortar with 0.5 gm. of phosphate-free charcoal (use more charcoal if needed to assure a clear solution). Add 10 cc. of 2 per cent. by volume acetic acid as the grinding is being done. Immediately mix thoroughly and filter through a dry filter into a test-tube. Each 0.2 cc. of this solution represents 0.1 gm. of sample. If the nitrate test cannot be made immediately, take the aliquot for the nitrate test and add an equal volume of 3 per cent. NaOH before allowing the extract to stand.

The extract usually is clear if stem or petiole tissue is used. Extract from leaf tissue may or may not be clear and the protoplasm of leaf cells may retain all the water, making it necessary to use more 2 per cent. acetic acid.

The succulence of the tissue varies the amount of extract secured, making it necessary to use the same weight of sample and the same volume of 2 per cent. acetic acid in making comparisons. The approximate parts per million of the nutrient can be found by making moisture determinations and allowing for the moisture of the sample. Comparisons can be made without doing this, since the amount of moisture in the same kind of tissue does not fluctuate enough to be significant in approximate analyses.

Nitrate test

This test should be made at once, to avoid enzymic reduction which will occur if the extract is allowed to stand. Put enough of the extract (usually 0.2–1.0 cc.) to give a good yellow nitrate color into a 25-cc. evaporating dish, add an equal volume of 3 per cent. NaOH, and evaporate to dryness without spattering or burning, over an alcohol lamp. Add 1 cc. of phenoldisulphonic acid (An) and cover all the residue well by rotating the dish. Let stand about 5 minutes and add about 10 cc. of water. Add clear 30 per cent. NaOH (Bn) slowly until a yellow color persists. Make up to 20 cc. and compare with the color standards for nitrate nitrogen.

PERMANENT COLOR STANDARDS FOR NITRATE NITROGEN.—Dissolve 0.3609 gm. of KNO_3 in distilled water and make up to 1000 cc. One cc. of this solution contains 0.05 mg. of nitrate nitrogen, and 0.1 cc. corresponds to 50 ppm. of nitrate nitrogen in the plant tissue, if 0.1 gm. of plant tissue is represented. If the extract from 0.2 gm. of tissue has been used, the color will represent 25 ppm.; if 0.4 gm. of tissue was required, the color represents 12.5 ppm., etc.

Prepare a series of standards as indicated in table I.

TABLE I

STANDARD NITRATE SOLUTION	NITRATE NITROGEN REPRESENTED IN GREEN TISSUE WHEN 0.1 GM. IS USED IN NITRATE TEST	
cc.	*ppm.*	
0.1	50	(Color varies from faint yellow at 50 ppm. to very strong yellow at 1000 ppm.)
0.2	100	
0.4	200	
0.8	400	
1.2	600	
1.6	800	
2.0	1000	

These standards will last indefinitely if kept in glass-stoppered tubes. They will keep in the test-tubes if stoppered with rubber stoppers and care is taken that the solution does not come into contact with the stoppers.

COLOR INTERFERENCE IN NITRATE TEST.—If the nitrate test cannot be read accurately because of interfering substances, grind 5 gm. of tissue with about 0.5 gm. of calcium hydroxide, add 10 cc. of 2 per cent. copper sulphate solution (2 cc. of 10 per cent. diluted to 10 cc. with water), mix well, allow to stand about 5 minutes, and filter (**4**). (The extract prepared in this way is not suitable for estimating phosphate phosphorus.) The $Cu(OH)_2$ usually clarifies the solution satisfactorily.

Phosphate test

Put sufficient plant extract (usually 0.2–2 cc.) to give a good color into a 10-cc. graduate. Make up to about 7 cc. with water. Add 1 cc. of molybdate solution (Ap) and 0.5 cc. of aminonaphthol sulphonic acid (Bp). Make up to 10 cc., mix, and allow to stand 10 minutes before comparing with the color standards for phosphorus.

TEMPORARY PHOSPHATE PHOSPHORUS COLOR STANDARDS.—Dissolve 0.1097 gm. of KH_2PO_4 in distilled water and make up to 2500 cc. One cc. of this solution contains 0.01 mg. of phosphate phosphorus and 0.1 cc. represents 10 ppm. of phosphate phosphorus in the plant tissue, if 0.1 gm. of plant tissue is represented. If 0.2 gm. of tissue has been used the color represents 5 ppm., etc.

Prepare a series of standards as indicated in table II. These standards must be made up at the same time as the unknown to secure proper comparison. The color deepens with standing.

PERMANENT PHOSPHATE PHOSPHORUS COLOR STANDARDS.—Measure out two 5 cc. portions of the phosphate standard solution prepared for the temporary standards. Develop the blue color as directed for the plant extract. Allow to stand 72 hours. Prepare a set of temporary standards. Dupli-

TABLE II

STANDARD PHOSPHATE SOLUTION	PHOSPHORUS IN GREEN TISSUE WHEN 0.1 GM. IS USED IN TEST	
cc.	*ppm.*	
0.1	10	(Color varies from very faint blue at 10 ppm. to very dark blue at 440 ppm.)
0.2	20	
0.5	50	
1.0	100	
2.0	200	
3.0	300	
4.0	400	

cate the colors developed by the temporary standards in 10 minutes by diluting appropriate amounts of the dark blue solution which has stood 72 hours with a clear solution made by adding 10 cc. of acid ammonium molybdate (Ap) and 4 cc. of aminonaphthol sulphonic acid (Bp) to 70 cc. of distilled water and making up to 100 cc. If the color is too light add a little more of the dark blue solution, or if it is too dark dilute with the clear acid molybdate solution until a set of standards made from the blue solution which has stood 72 hours exactly duplicates the temporary set. The permanent set usually will keep for several days, but for accurate work it is best to make up fresh standards at the time the tests are made.

Potassium test

Put sufficient plant extract (usually 0.5–2 cc.) to cause an appreciable reduction of color of the potassium reagent into a 25-cc. evaporating dish and evaporate almost to dryness, without spattering or burning. Add 0.3 cc. of sodium cobaltinitrite (Ak), cover all the residue by rotating the dish, and allow to stand about 5 minutes. Make to 10 cc., in a 10-cc. graduate, with sodium nitrite solution (Bk). After the precipitate settles, compare with the color standards for potassium. The comparison may be made immediately by filtering off the precipitate on a dry filter. The lighter the color the greater the amount of potassium. If the solution is nearly colorless, another test should be made, using less plant extract.

TEMPORARY POTASSIUM STANDARDS.—Dissolve 1.2929 gm. of KNO_3 in distilled water and make up to 250 cc. One cc. of solution contains 2.0 mg. of potassium and 0.3 cc. represents 600 ppm. of potassium in the plant tissue, if 1.0 gm. of plant tissue is represented. If 2.0 gm. of tissue has been used the color represents 300 ppm., etc.

Prepare a series of standards as indicated in table III. These standards are good only for the day they are made; the color fades slowly on standing over night.

TABLE III

STANDARD POTASSIUM SOLUTION	POTASSIUM REPRESENTED IN GREEN TISSUE WHEN 1.0 GM. IS USED IN TEST	
cc.	*ppm.*	
0.00	0	(Color varies from dark brown color at 0 ppm. to faint yellowish brown at 3000 ppm.)
0.30	600	
0.60	1200	
0.90	1800	
1.20	2400	
1.35	2700	
1.50	3000	

PERMANENT POTASSIUM STANDARDS.—When ferric chloride is dissolved in concentrated hydrochloric acid, a permanent color is produced which is almost the same as the sodium cobaltinitrite color. Make a strong solution of $FeCl_3$ in hydrochloric acid. Duplicate the color of the temporary potassium standards by diluting appropriate amounts of the $FeCl_3$ solution with hydrochloric acid. These solutions will keep indefinitely.

TABLE IV

COMPARISON OF LABORATORY AND FIELD TESTS FOR NITRATE AND PHOSPHATE, MADE ON THE SAME MATURE TOMATO PETIOLES (PPM. OF GREEN PLANT TISSUE)

PLANT	TREATMENT	NITRATE N		PHOSPHATE P		NOTES ON APPEARANCE OF PLANT
		LABORATORY	FIELD	LABORATORY	FIELD	
		ppm.	*ppm.*	*ppm.*	*ppm.*	
1	Check; a rather poor red soil	980	1000	Trace	Trace	Fairly green and large; fruit set poor
2	400 lb. of sodium nitrate per acre	1666	1600	266	200	Green, large plants; fruit set fair
3	20 tons manure and 1 ton $Ca(OH)_2$ per acre	1500	1300	300	250	Quite vegetative and large; fruit set fair
4	400 lb. of sodium nitrate per acre	1666	1600	266	200	Same as plant no. 2
5	4 tons of straw per acre	Trace	None	660	500	Stunted, yellow plants; fruit set good
6	40 tons of manure, 1 ton $Ca(OH)_2$, 0.5 ton Na_2CO_3	1666	1500	600	400	Very large; vegetative plants with good set of large fruits

Results

COMPARISON OF FIELD AND LABORATORY TESTS

Table IV shows quantitative results obtained by the laboratory procedure (**4**, **5**) and the amounts estimated by the field tests in samples from the same plants. The two sets of determinations agree well, considering that the field test is approximate. The nitrate determinations check quite closely, while the laboratory values for phosphate are slightly higher than those obtained by the field test.

Table IV also shows a good correlation between fruit set and phosphate phosphorus content, as well as between vegetation and nitrate nitrogen content. The appearance of the plant cannot always be relied upon, however, since some disease or nutritional factors other than lack of nitrate may cause a yellowish color. Many other factors besides lack of phosphorus may cause a poor set.

RESULTS OF FIELD TESTS ON DIFFERENT PLANTS

In order to determine whether the tests would work on different plants, determinations were made on plants of several kinds. The results are presented in table V. In a few of these, interference by carbohydrate was experienced in making the nitrate test on the charcoal extract. When $Ca(OH)_2$ and copper sulphate were used as the clarifying agents, the difficulty was overcome. The kinds of tissue in which this occurred were fresh asparagus tips, green onions, and small peach fruits.

TABLE V

RESULTS OF FIELD TESTS (PPM. OF GREEN TISSUE)

PLANT	N	P	K
	ppm.	*ppm.*	*ppm.*
Peas (stunted), stems	Trace	700	1000
Peas (vigorous), stems	800	300	2500
Rhubarb (vigorous), petiole	150	800	500
Coleus (vigorous), stem	2000	350	
Onion (vigorous), stem	250	300	700
Asparagus (stem tissues of tips)	Trace	600	900
Peach, petioles	600	1000	1500
Raspberry (vigorous), petioles	300	5	800
Raspberry (mosaic yellow), petioles	50	10	400
Potted tomato plant (root-bound and yellow)	50	30	600
Potted tomato plant (green and stocky)	400	15	400
Young corn plants (vigorous)	700	10	1800
Young peach fruits	100	900	600

INTERPRETATION OF FIELD TESTS

As yet, not enough tests have been made to determine the exact concentrations of nutrients which should be maintained in the various plants to obtain optimum yields. However, the concentration found in vigorous, high-yielding plants should be a guide to the amount of nutrient which should be present. For instance, table IV shows that the vigorous tomato plants possessing a good set of fruit contained about 1600 ppm. of nitrate nitrogen, 600 ppm. of phosphate phosphorus, and 3800 ppm. of potassium. The nitrated plants contained 1600 ppm. of nitrate nitrogen and looked healthy, yet the fruit set was not good. The tests revealed that only 200 ppm. of phosphate phosphorus was present. The inference is that it would pay to add available phosphorus. Field tests can thus be used to reveal nutrient deficiencies and to form a basis for intelligent fertilizer application to supply the specific need of a particular crop.

Field tests on the plant indicate what soil nutrients are available to the plant, and enable the determination of the optimum ratios and concentrations of nitrate, phosphate, and potassium for each kind of plant. The interference experienced in soil tests with certain soils is eliminated. The tests may be performed rapidly in the field, dispensing with the necessity of a laboratory and hours of laboratory routine.

UNIVERSITY OF KENTUCKY,
LEXINGTON, KY.

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SEASONAL CHANGES IN THE COMPOSITION OF THE INSOLUBLE NITROGEN FRACTION IN THE CURRENT YEAR'S SHOOTS OF BARTLETT PEAR

A. S. MULAY

(WITH TWO FIGURES)

In a previous paper (**1**), it was shown that there were considerable variations in the quantity of insoluble nitrogen, both in bark and wood, during the growth cycle of pear shoots. It was also shown in another paper (**2**) that seasonal variations in the water-soluble nitrogen fraction in pear shoots are not only quantitative but qualitative also. It was of interest to find how the insoluble nitrogen fraction, representing mainly cytoplasmic proteins, behaved in this respect. As a convenient way of partially answering this question, a Van Slyke fractionation of the insoluble nitrogen was undertaken, although it was apparent that the constancy of results by this method would be no proof of the constant composition of this fraction.

Experimentation

Current year's shoots were separated into bark and wood, dried at 50° C., and ground to 100 mesh. This powder was shaken with water at room temperature for two hours, and the residue separated from the soluble extract by centrifuging and washing. This residue was dried at 50° C. and powdered. The amount of total nitrogen in this residue is called insoluble nitrogen. The material used and the procedure followed are fully described in the paper (**1**) on the seasonal variations in soluble and insoluble nitrogen. A 25-gram portion of this residue was extracted with benzene and alcohol in the order named and the nitrogen determined in these extracts. The extracted sample was gently boiled with 20 per cent. hydrochloric acid for 48 hours under a reflux condenser. The hydrolysate was filtered under suction on hardened filter-paper and the residue washed with hot water until free from chlorides. The filtrate and the washings were evaporated nearly to dryness under reduced pressure. This concentrate was taken up with hot water, made up to 500 cc., and total nitrogen determined on an aliquot. The remainder was carried through the amide and other determinations as described elsewhere (**2**). Tables I and II summarize the results obtained.

Discussion

The insoluble nitrogen of the bark (fig. 1) contains 40–50 per cent. amino nitrogen, nearly 20 per cent. basic nitrogen, 10–15 per cent. melanin nitrogen, 7–9 per cent. amide nitrogen, 4–6 per cent. humin nitrogen, 5–10 per cent. rest nitrogen, 2 per cent. alcohol-soluble nitrogen, and about 0.1

TABLE I

INSOLUBLE NITROGEN FRACTIONATION
AS PERCENTAGE OF INSOLUBLE NITROGEN
BARK

DATE COLLECTED	BENZENE EXTRACT	ALCOHOL EXTRACT	MELANIN	NITROGEN IN HYDRO-LYSATE	AMIDE	HUMIN	BASIC	AMINO	REST
Sept. 16	0.1	2.0	16.0	82.0	8.1	4.4	17.4	44.5	7.8
Oct. 21	0.1	1.7	16.3	82.2	8.1	3.6	17.2	43.4	10.3
Dec. 7	..	2.1	14.3	83.6	7.2	5.1	20.9	40.9	9.6
Jan. 15		2.2	9.7	88.1	7.9	6.3	23.2	46.8	4.2
Feb. 11	0.2	2.2	9.7	89.2	8.4	5.6	22.7	47.5	4.2
Mar. 18		2.0	12.9	85.3	8.2	4.3	24.6	44.6	3.6
Apr. 18	0.3	2.4	10.2	87.5	9.2	5.1	20.5	46.8	7.3
May 13		2.2	10.9	86.8	9.1	6.5	19.9	44.9	6.5
June 24	0.3	2.0	5.3	92.8	8.7	4.0	22.9	51.4	5.9
July 15	0.2	2.0	11.6	86.5	8.4	8.6	19.3	49.0	1.1
Aug. 19	0.1	2.0	10.8	87.2	8.6	4.3	20.8	52.0	1.4
Sept. 19	0.2	2.0	12.1	80.5	7.8	7.7	18.1	49.3	5.6
Oct. 15		2.0	11.1	87.1	8.2	6.4	20.0	46.7	5.7
Nov. 17	0.2	2.0	15.4	80.7	7.8	7.9	18.3	43.4	4.5
Jan. 18	0.2	2.1	9.6	87.5	8.2	5.6	19.6	45.1	9.0

TABLE II

INSOLUBLE NITROGEN FRACTIONATION
AS PERCENTAGE OF INSOLUBLE NITROGEN
WOOD

DATE COLLECTED	BENZENE EXTRACT	ALCOHOL EXTRACT	MELANIN	NITROGEN IN HYDROLYSATE	AMIDE	HUMIN	BASIC	AMINO	REST
Sept. 16	0.4	7.3	10.9	81.3	13.3	3.2	10.8	...	.....
Oct. 21	0.3	8.5	11.9	79.2	10.7	4.0	8.3	40.5	15.7
Dec. 7	0.2	7.2	12.2	80.6	12.4	4.0	11.9	36.0	16.4
Jan. 15	0.2	6.9	12.7	82.6	10.8	4.9	11.9	43.0	12.0
Feb. 11	0.2	7.8	12.6	79.5	11.4	3.6	11.2	39.5	13.5
Mar. 18	0.2	7.2	12.9	80.0	12.0	4.9	12.2	39.6	11.5
Apr. 18		7.0	14.0	78.0	11.1	0.2	13.3	41.4	13.0
May 13		5.7	13.8	80.6	11.2	1.2	14.2	40.2	13.7
June 24		6.4	12.2	81.4	10.6	4.8	14.6	42.1	9.0
July 15		8.6	11.2	80.8	10.5	7.3	13.2	39.3	10.5
Aug. 19		9.8	18.1	72.1	9.3	3.1	15.1	35.0	9.2
Sept. 19		8.2	22.7	70.5	9.4	5.1	13.6	33.5	9.2
Oct. 15	0.2	11.7	15.6	73.0	8.7	1.5	16.8	32.7	13.2
Nov. 17	0.2	8.7	17.3	74.1	8.5	0.4	13.5	37.7	14.1
Jan. 18	0.2	6.0	14.9	79.0	8.9	2.8	13.0	34.5	20.0

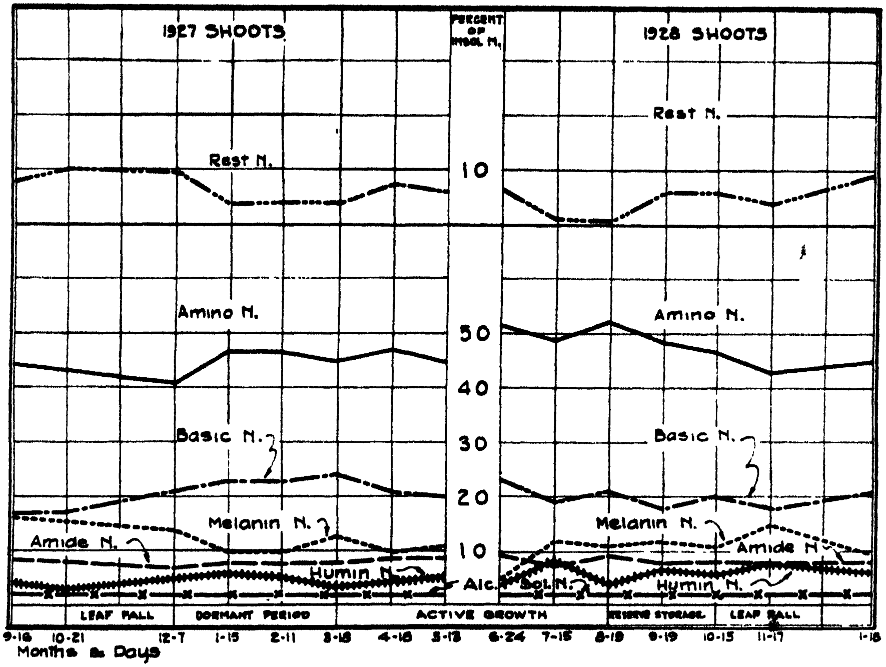

FIG. 1. Seasonal changes in composition of the insoluble nitrogen fraction in the bark of the current year's shoots of Bartlett pear.

per cent. benzene-soluble nitrogen. This composition seems to remain more or less constant throughout the year, except that at the beginning of the growing season there is a slight rise in amide and humin nitrogen at the cost of basic and rest nitrogen; and as growth proceeds there is a slight fall in amino nitrogen with a parallel rise in rest nitrogen.

The insoluble nitrogen of the wood is composed of about 40 per cent. amino nitrogen, 12 per cent. basic nitrogen, 14 per cent. melanin nitrogen, 10 per cent. amide nitrogen, 12 per cent. rest nitrogen, 4 per cent. humin nitrogen, 8 per cent. alcohol-soluble nitrogen, and 0.1 per cent. benzene-soluble nitrogen. The composition of the insoluble nitrogen of the wood, like that of the bark, is rather constant, except that from June to August, 1928, there was a marked fall in amino nitrogen and a rise in melanin nitrogen. There is a tendency for rest nitrogen to rise after active growth stops.

When compared with bark, wood seems to be especially rich in alcohol-soluble and rest nitrogen and poorer in basic nitrogen.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

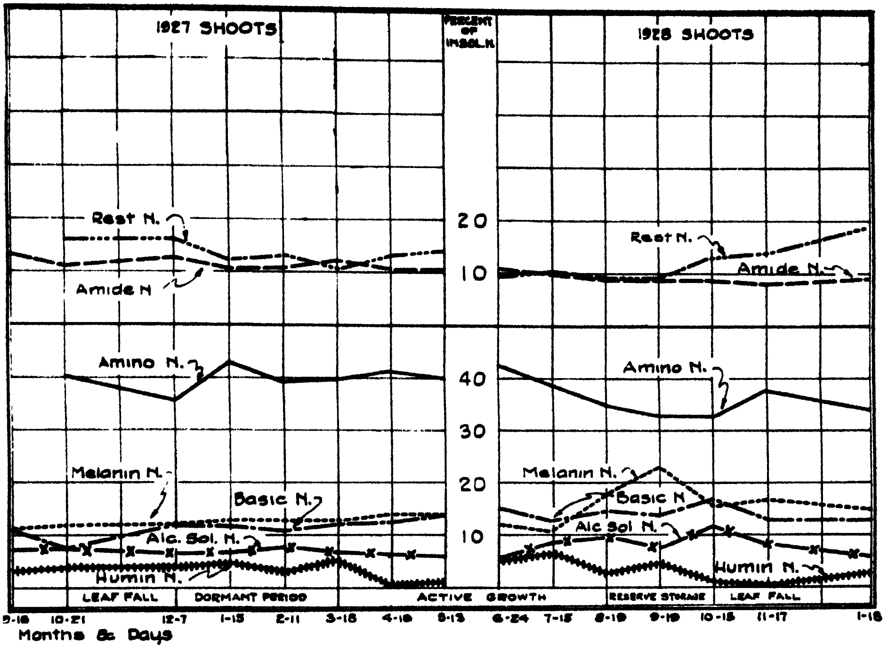

FIG. 2. Seasonal changes in composition of the insoluble nitrogen fraction in the wood of the current year's shoots of Bartlett pear.

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CHLOROSIS OF TREES[1]

EDMUND BURKE

(WITH ONE FIGURE)

Introduction

Chlorosis is a condition of the leaf in which chlorophyll fails to develop normally. **Bourcart** (1) states that this pathological condition is known as jaundice when caused by want or excess of water, and chlorosis when it is due to lack of iron or potash. An excess of carbonate of lime in the soil, insufficient nourishment, defective condition of the roots, a pathological condition of the whole tree, are some of the many causes which may produce chlorosis. When due to lack of iron, chlorosis has some analogy to the anemia of man.

Much has been published on the causes and treatment of chlorosis. No attempt is made to cite all the literature published, but a few citations are given to indicate the extent of its occurrence, and its treatment.

Bourcart (1) states that an excess of carbonate of lime is the predominating cause of chlorosis of trees with seed pips, such as the pear, and more especially of the vine. He recommends green vitriol for both trees and vineyards. **Kelley** (7) found that an excess of manganese in the soil was responsible for the chlorotic condition of pineapples, as well as some other plants in Hawaii. **Johnson** (6) confirmed **Kelley**'s finding and found that chlorotic pineapples were restored to normality by spraying with an iron sulphate solution.

Wallace and **Mann** (8) found considerable difference in the chemical composition of the ash of chlorotic and normal fruit trees. The percentage of ash in the dry matter, with one exception, was higher in the chlorotic than in the normal leaves.

The Chemistry Department of the Idaho Agricultural Experiment Station (5) associates the chlorosis of trees and shrubs with high calcium carbonate soils even though a considerable amount of iron is present. **Giles** (3) found that the chlorosis of pineapples in Porto Rico was the result of malnutrition, caused by an excessive amount of calcium carbonate in the soil. **Giles** and **Carrero** (4) report that chlorotic rice plants may be restored to their normal color by spraying with ferrous sulphate.

Burgess and **Pohlman** (2), in studying the cause of citrus chlorosis, found that improper irrigation was a factor. Spraying the trees with an iron salt solution did not give beneficial results.

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[1] Contribution from Montana State College, Agricultural Experiment Station, Paper no. 14, Journal series.

It is evident, from the literature cited, that numerous plants become chlorotic when grown in soils high in calcium carbonate, or manganese, or when improperly irrigated.

The first investigation of chlorosis at the Montana Station dates back to the spring of 1908, at which time the Departments of Chemistry and Botany treated a chlorotic cottonwood tree, growing on the College campus, with a 0.25 per cent. solution of ferrous sulphate. The tree was about ten inches in diameter and four liters of the iron solution were injected into the trunk. Improvement was rapid and the tree had fully recovered by the following spring. Since that time several cottonwood trees have been treated with a solution of ferrous sulphate with equally good results. A slight burning of the leaves occurred when a 0.5 per cent. solution was used and as much as four liters applied to trees eight to ten inches in diameter.

An investigation of apple trees was started in 1914 when the writer's attention was called to a condition affecting the orchards in certain sections of the Bitter Root Valley, the fruit section of the State. At the time of inspection the trees were making poor growth and the leaves were small and yellowish in color. It was found, on taking soil samples near the trees showing abnormal conditions, that the subsoil consisted quite largely of sand and gravel of various sizes cemented together by calcium carbonate. It was almost impossible to go through this formation with a pick or a crowbar. The average analyses of several samples gave the following results: First foot, nitrogen, 0.095 per cent., calcium 8.05 per cent., phosphorus 0.105 per cent., and potassium 0.29 per cent.; second foot, nitrogen 0.057 per cent., calcium 14.62 per cent., phosphorus 0.083 per cent., potassium 0.20 per cent., and iron 0.35 per cent.

The analyses indicate that the soil was fairly well supplied with nitrogen and phosphorus, with an abnormally large supply of calcium and a rather low amount of potassium. The percentage of iron was considerably less than is found in fertile soils of the state.

No further investigation was made until the fall of 1922, when our attention was again called to this trouble by the State Horticulturist, who pointed out that chlorosis in the Bitter Root Valley might prove as injurious to that fruit section as had the fruit-tree leaf-roller, which had just made its appearance and was doing considerable damage. It was found that the greatest injury was occurring in a high-lime soil area.

In the orchard selected for conducting the experiments many of the trees were not only failing to make normal growth, but several were dead. The leaves on the chlorotic trees were small and yellow, and the trees had made little or no growth. The poor condition of the trees had been attributed by some people to high water-table and a water-logged condition, while others thought it was the result of too much alkali in the soil. However, a hole dug five feet deep near a chlorotic tree failed to reach the water-table and an analysis of the soil revealed the presence of very little alkali salts, not enough to cause tree injury. An average analysis of the second foot of soil showed a calcium content of 14.53 per cent. and 1.0 per cent. of iron. The samples were not analyzed for nitrogen, phosphorus, and potassium, since we believed that they would not vary materially from the analyses given.

An inspection of this orchard, made a few days after it had been irrigated, showed an apparent water-table at a depth ranging from two to three feet. This condition, while only temporary, undoubtedly had the effect of decreasing the supply of available nitrogen, which would result in lightening the color of the leaves.

Treatment of trees

After making a study of the soil and trees it was decided that the chlorotic condition was probably caused by too much lime in the soil, and that treatment with iron should prove beneficial. The first treatments were made in August, 1922, when 500 cc. of a 0.25 per cent. solution of ferrous sulphate were injected into each of two trees with yellow leaves that had failed to make a normal growth. A hole, one-fourth inch in diameter, was bored into the sap wood of the trunk of the tree, into which an L-shaped gas pipe was fitted. One end of a rubber tube was slipped over the end of the gas pipe and the other end was attached to a wide-mouthed bottle containing iron sulphate solution. The bottle was inverted and hung to a branch of the tree about four feet above the gas pipe in the trunk. To admit air into the bottle a U-shaped glass tube was used, one end being pushed through a rubber stopper to the bottom of the bottle and the other end projecting to a little above the bottom on the outside. By this method the iron solution was injected into the tree. Two other trees were treated by digging a circular trench about one foot deep and four feet from the trunk. Five pounds of ferrous sulphate were scattered in the trench, after which it was covered with earth and watered. Another set of two trees was sprayed with a 0.5 per cent. solution of ferrous sulphate. Several other trees showing a decidedly chlorotic condition were treated with a complete fertilizer to determine if a lack of plant food might be the cause.

Results

An inspection made in the fall showed that neither the soil application nor the spray treatment had improved the chlorotic condition of the trees. The injection of ferrous sulphate had only slightly improved the color of the leaves formed at the time of treatment, but the leaves which had formed after treatment were of a green color and normal in appearance. Figure 1 gives a fair idea of the difference in the appearance of the leaves before and after treatment. The upper three leaves were formed after treatment.

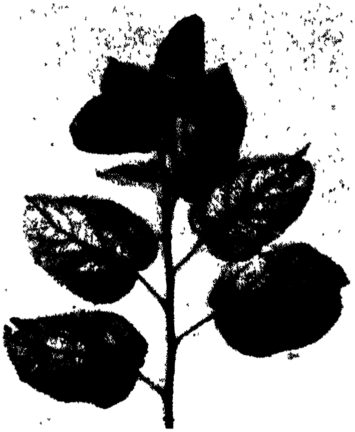

FIG. 1. Leaves of apple, showing recovery from chlorosis. The upper three leaves produced after treatment.

An inspection made the following June showed no beneficial effect from the spray, soil or fertilizer treatment, while the two trees into which ferrous sulphate had been injected were normal in appearance and remained so.

From these results it was evident that a solution of ferrous sulphate injected into a tree would restore the leaves to their normal color. Another test was undertaken in May, 1924, when twelve trees with yellow leaves were treated with ferrous sulphate or ferric nitrate. In some cases the iron salts were injected into the trunk of the tree for the purpose of restoring the whole tree to normal condition, while in other instances, the injection was made into one branch of the tree so that a comparison could be made between the color of the leaves on the treated and the untreated branches. In addition to injecting an iron solution into the tree, a one-half inch hole, sloping downward, was bored into the tree and either ferrous sulphate or ferric nitrate salts inserted and water added until the salts were dissolved. Another yet simpler method of supplying the iron, one that any orchardist could use, was also tried, *viz.*, driving iron nails into the trees. Heavy finishing nails, about one to one and one-fourth inches long, were used for this purpose. Care was used in driving the nails into the tree to avoid injuring the bark, and a nail-set was used to drive the nails so that the heads would be completely under the bark. In the case of branches some eight or ten nails were driven in. Where the nails were driven into the trunk as many as fifteen or twenty were used. The nails did not give as rapid

TABLE I

CONDITION OF TREES, TREATMENT AND RESULTS

TREE NUMBER	CONDITION OF TREE	TREATMENT	RESULTS
1.	All leaves were yellow, general condition bad	Worst branch treated with 2 gm. $Fe(NO_3)_3$ crystals	Some improvement during summer. Following year the leaves were all yellow. Tree pulled
2.	Leaves were yellow	2 gm. $FeSO_4$ crystals put into hole in trunk	Recovery was complete
3.	Leaves were yellow	3 gm. $Fe(NO_3)_3$ crystals put into hole in trunk	Recovery was complete
4.	Decidedly chlorotic	Worst branch treated with 3 gm. $Fe(NO_3)_3$ crystals	Branch recovered, remainder of tree remained chlorotic
5.	Part of the leaves were yellow	30 cc. of 12.5 per cent. solution of $Fe(NO_3)_3$ injected into trunk	Recovery was complete
6.	Decidedly chlorotic	5 gm. $FeSO_4$ crystals put into one branch. One year later, iron nails were driven into trunk	Treated branch recovered first. Last inspection found an almost complete recovery
7.	Decidedly chlorotic	Worst branch treated with 3 gm. $FeSO_4$ crystals. One year later iron nails driven into trunk	Treated branch recovered first. Balance of the tree recovered slowly, but completely
8.	Decidedly chlorotic	500 cc. 0.25 per cent. solution of $Fe(NO_3)_3$ injected into one branch. One year later iron nails driven into trunk	Slight improvement the first year. Last inspection found the tree still chlorotic
9.	Worst physical condition of any of the trees treated	First, 5 lb. $FeSO_4$, second, 500 cc. 0.25 per cent. solution $FeSO_4$ injected into one branch. Third, complete fertilizer added to soil. Fourth, 5 gm. $FeSO_4$ crystals put into hole in trunk. Fifth, iron nails driven into trunk	Slow, but complete recovery
10.	Decidedly chlorotic	500 cc. 0.25 per cent. solution of $FeSO_4$ injected into one branch. One year later, iron nails driven into trunk	Recovery was complete
11.	All leaves were yellow	2 gm. $FeSO_4$ crystals put into trunk	Recovery was complete
12.	All leaves were yellow	500 cc. 0.25 per cent. solution of $FeSO_4$ injected into one branch	Leaves on treated branch became normal, those on remaining branches remained chlorotic

action as when the iron was put into the tree in solution, yet the final results were as satisfactory. The accompanying table describes the condition of each tree at the time of treatment, May 20, 1924, the kind of treatment and the results obtained.

In addition to the trees described in the table, four trees were selected for treatment with iron nails only. On June 1, 1925, iron nails were driven into the trunks of the trees. The leaves on all of the trees were yellow at the time of treatment. A slight improvement was noted when inspected on August 18, 1926. On June 21, 1927, when last inspected, recovery was complete.

Summary

Cottonwood trees are susceptible to chlorosis, which can be overcome by treatment with iron salts.

Apple trees grown in high lime soils are subject to chlorosis. The lime evidently prevents the iron in the soil from becoming available. The fact that, in most cases, iron salts restored trees to normality indicates a lack of *available* iron in the soil.

Spraying with a 0.5 per cent. solution of ferrous sulphate, or applying ferrous sulphate to the soil, did not improve the chlorotic condition of the trees.

Treatment internally with iron salts, both as dilute solutions and as solid salts, gave beneficial results.

Iron nails driven into the trunks of four trees gave beneficial results. The recovery of chlorotic trees treated with iron nails was not so rapid as when treated with iron salts, although it seemed to be as effective. The ease of application should appeal to orchardists.

MONTANA AGRICULTURAL EXPERIMENT STATION,
BOZEMAN, MONTANA.

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SIERGEI PAVLOVITCH KOSTYTCHEV
1877–1931

BRIEF PAPERS

SIERGEI PAVLOVITCH KOSTYTCHEV

(WITH ONE PLATE AND ONE FIGURE)

On August 31, 1931, there died in Crimea, Russia, the prominent plant physiologist, S. P. KOSTYTCHEV. His death came as a shock to his friends abroad, especially to those who attended in 1930 the Second International Soil Science Congress and who have learned to know personally this brilliant investigator.

KOSTYTCHEV was born in St. Petersburg in 1877, the son of a Professor, a prominent soil scientist. In 1896 he entered the local University, specializing in botany and especially in plant physiology, under D. I. IVANOVSKI. Upon graduation in 1900, he left for Western Europe, studying plant chemistry with E. SCHULZE in Zürich and with A. KOSSEL in Heidelberg. On his return to Russia in 1903, he became attracted by the brilliant investigations of V. I. PALLADIN, on plant respiration, and became the outstanding representative of PALLADIN's school in Russia. His Master's thesis in 1907 dealt with "Investigations on the anaerobic respiration of plants," and his Doctor's thesis in 1910 on "Physiologo-chemical investigations of plant respiration." He was at first assistant in the Academy of Medicine in St. Petersburg; in 1910 he was appointed professor at the Technological Institute and in 1914 professor of plant physiology at the University. In 1923 he was elected a member of the Russian Academy of Sciences.

He is best known for his investigations on the rôle of anaerobic processes in normal respiration. During the germination of peas, in the absence of oxygen, alcohol was accumulated, while the products of incomplete fermentation of sugar by yeasts added to wheat seedlings were completely oxidized to CO_2. His later work was centered primarily upon the mechanism of alcoholic fermentation, especially the intermediary fermentation products during the last stages of the process, and the rôle of acetaldehyde in this process. His investigations of carbon assimilation by different types of plants as influenced by environmental conditions, of the rôle of zymase in alcoholic fermentation, of the relation of fermentation to respiration in yeasts and filamentous fungi, of the mechanism of nitrogen assimilation by fungi, and of the formation of ammonia in the process of nitrogen fixation by *Azotobacter*, have contributed materially to the advance of our knowledge of the physiology of higher plants and lower organisms. More recently, KOSTYTCHEV was placed in charge of the Division of Agricultural Microbiology at the Institute of Experimental Agronomy and later was made the chief of a new Institute of Microbiology of the Lenin Agricultural

FIG. 1. A group of investigators interested in the rôle of micro-organisms in soil processes and plant growth at the meeting of the Third Commission of the International Congress of Soil Science at Stockholm in 1929.

Academy, where he surrounded himself with a considerable number of young investigators, working on numerous problems in the field of agricultural microbiology.

He is best known in this country for his books on "Plant respiration" and "Plant physiology." He took an active part in organizing the program and the meetings of the Third Commission on Soil Microbiology in 1929, in Stockholm, and of the Second International Soil Science Congress in 1930, in Leningrad and Moscow. As Vice-President of this commission, he contributed greatly to making the meetings successful. His knowledge of foreign languages, his charming personality, his deep interest in his work, made him beloved and respected by all who came in contact with him.—SELMAN A. WAKSMAN, *New Jersey Agr. Exp. Sta., New Brunswick, N. J.*

SOME PHOTOPERIODIC AND TEMPERATURE RESPONSES OF THE RADISH

(WITH ONE FIGURE)

In connection with their studies on photoperiodism, GARNER and ALLARD (2) pointed out that the formation of tubers commonly results from excessively short days. The present brief experiment was performed to detect what influences, if any, temperature as well as length of day exerts upon the development of the storage organ of the radish.

Seeds of the radish, *Raphanus sativus,* of the Earliest Carmine Turnip variety, were planted in four pots on June 29, 1931. They were placed under the four following conditions: 25° C. and long day, 25° C. and short day, 15.5° C. and long day, 15.5° C. and short day. The temperatures were maintained in controlled glass chambers. The long day was the natural day length, about 15 hours. The short day was 7.5 hours (9 A. M. to 4:30 P. M.), the pots being covered with cardboard boxes the rest of the time. Since the temperature within the boxes did not rise more than 1.5° above the temperature without, the difference was considered negligible.

The percentage of thickened hypocotyls at low temperature was approximately the same under the two light conditions after about five weeks, but those with the shorter day length were more uniform and slightly smaller. At the higher temperature the results were different: there were fewer thickened hypocotyls under short-day conditions than under long-day conditions. It was striking that while thickening was good with both light periods at low temperature, it was very poor with both light periods at high temperature. In the latter case the hypocotyl tended to assume a procumbent position; also the foliage was larger than at low temperature. It is pointed out by GARNER and ALLARD (3) that short-day illumination checks vegetative growth and causes an increase in tuber formation. From the present results it would seem that temperature must also be taken into account.

Microchemical tests (1) at the end of the day (4:30 P. M.) showed that all the thickened hypocotyls contained a little fructose and a great abundance of glucose (Fehling's solution). This is in accordance with NIGHTINGALE's report (4) that the radish stores sugars, not starch, in the storage organ. The small thickened hypocotyls, however, grown at high temperature and with a long day, showed an abundance of very small starch grains in the parenchymatous tissues (iodine potassium iodide). RIOLLE (5) reports that some varieties of radishes, especially Chinese ones, may contain a considerable amount of starch; but she also reports that very few French and Japanese varieties ever contain any starch, and then only very little. So it would appear here that a variety which does not form starch under

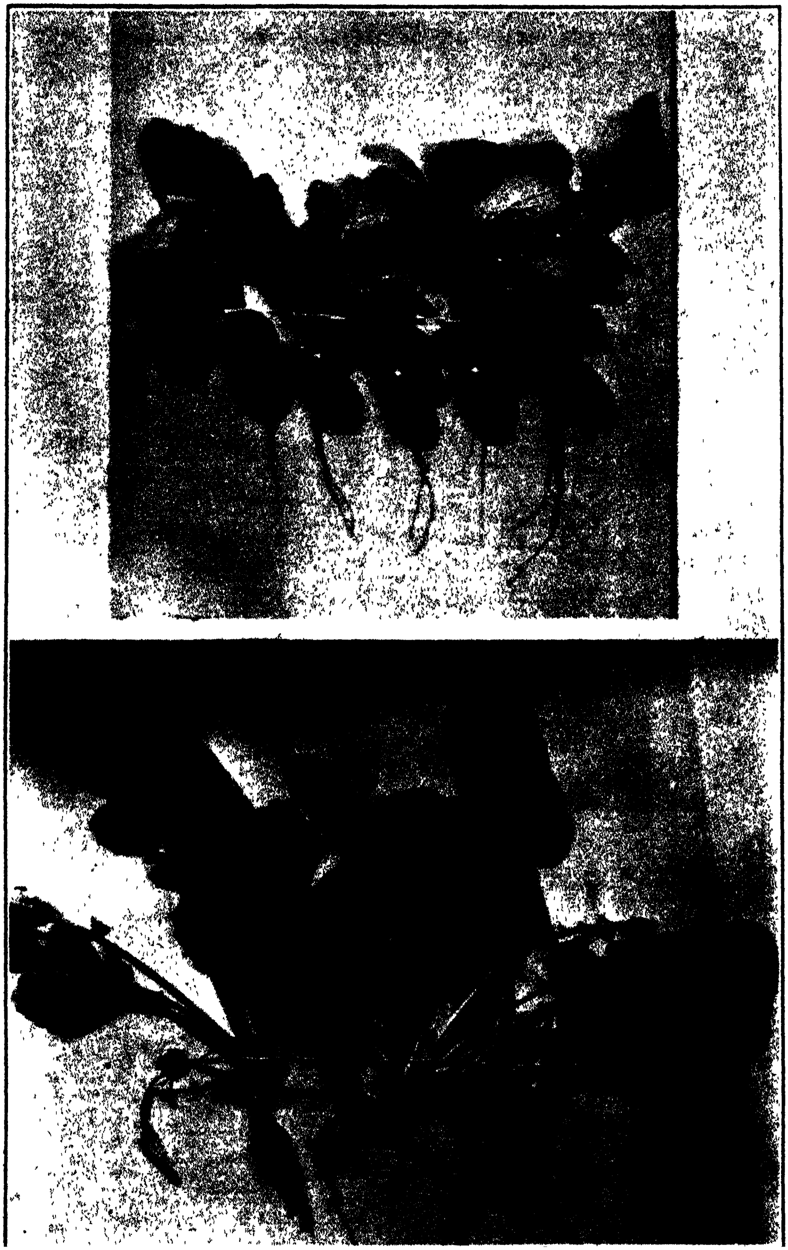

Fig. 1. Radishes grown under short-day conditions: above, low temperature; below, high temperature.

ordinary circumstances does do so under certain extreme conditions. May not this unexpected and unusual condition indicate that temperature, at least beyond the range normal for the radish, influences the form in which the carbohydrates are stored?

This experiment was kindly suggested by Dr. H. E. HAYWARD and guided with constant interest by Dr. C. A. SHULL.—THORA M. PLITT, *University of Chicago.*

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TOXICITY OF ROOT EXCRETIONS

(WITH ONE FIGURE)

From ancient times the belief has persisted that certain species of plants may give off through their roots excretions which are toxic to the roots of other plants. Although the evidence for this idea has never been very strong, it has been kept alive in the literature by occasional observations and investigation of alleged cases of the phenomenon. PICKERING'S[1] studies of the influence of grass roots upon the behavior of the apple are well known. The discovery of dihydroxystearic acid in wheat-sick soils was at one time interpreted as a case of toxic excretion. More recently FLETCHER[2] reported the extreme sensitivity of *Sesamum indicum* to the root excretions of *Andropogon sorghum* (*Sorghum vulgare*), and SEWELL[3] found that the roots of *A. sorghum* injured succeeding crops of wheat. He was able to obtain an extract from the soil in which the *Sorghum* was growing which was toxic to the wheat plant if the extract were prevented from becoming oxidized.

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In some of these cases the alleged toxicity has been reinterpreted in terms of competition of root systems for nutrients such as nitrogen in the soil; in others, the toxicity can be interpreted in terms of the products of root decay in the soil. The case of *Sesamum indicum* mentioned by FLETCHER seemed very extraordinary. His photographs indicate a profound effect upon the growth of *Sesamum,* and he concludes that *Sesamum* cannot grow to maturity within 20 inches of *A. sorghum.* It is practically certain that not all of the influential factors were under control in FLETCHER's field tests, if indeed any of them were. But the stunted growth was so remarkable that the writer had long desired to see the results of the *Sorghum* root excretions upon the *Sesamum* plant. The opportunity to make such studies was not presented until very recently. Through the courtesy of Prof. G. S. KULKARNI, and Prof. V. G. GOKHALE, of the Agricultural College, Poona, India, seeds of five varieties of the Indian jowar (*S. vulgare*) were obtained (Bedari, Fulgar, Gidgap, Maldandi, and Perio), as well as seeds of both the white- and black-seeded varieties of *Sesamum indicum.*

These seeds were first grown in pot cultures in the greenhouses of the Oregon State College[4] during the spring of 1931. Each of the varieties of *Sorghum,* and the two varieties of *Sesamum,* were grown alone as controls. There were five experimental cultures in which each variety of *Sorghum* was grown along with black sesame, and five in which white sesame accompanied the sorghums.

The soil used was a light sandy mixture which proved to be too light for very vigorous growth, but in every case the variety of *Sorghum* grew in association with the *Sesamum* plants without causing any apparent injury to the latter. At the end of about nine weeks the pots were examined for root growth. It was found that the roots of the *Sorghum* and *Sesamum* plants were growing together most intimately, without the slightest sign of abnormality of root development in the *Sesamum.* There was nothing to suggest any toxic action on the part of the *A. sorghum* roots.

After returning to the University of Chicago, a bed of *Sorghum* and *Sesamum* was planted about July 1, 1931, in one of the ground-level beds of the Botany greenhouses. Five rows of sorghum, one of each variety, were planted about 16 inches apart, running north and south. Between each two rows of *Sorghum* was planted a row of *Sesamum indicum,* white or black, two rows of each being used. These were planted thus about 8 inches from the sorghums. The seeds germinated very promptly and the plants grew luxuriantly in the closest association. Figure 1 shows a photograph of the plants when about half grown. There were no indications of toxicity of the roots of *A. sorghum* (*S. vulgare*) for the roots of *Sesamum indicum*. The plants continued to grow until they reached the blooming stage together, during the first week in October. Plants of both species were blossoming normally within 8 inches of each other. The sorghums at their best were over 3 meters tall, and the sesame plants more than a meter tall. It was necessary to thin the sorghums to prevent shading of the sesame plants, and to remedy lodging of the sorghums from low light intensity which was aggravated by the thick planting.

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[4] I am indebted to Professor H. P. BARSS and Dr. W. M. ATWOOD, and to the Administrative Officers of the Oregon State College, for many courtesies extended to me in connection with this and other problems.

FIG. 1. Three Indian varieties of *A. sorghum*, Gidgap, Fulgar, and Bedari, with *S. indicum*, black-seeded, growing between them.

The results failed entirely to confirm the alleged toxicity of the sorghums for *S. indicum*. Whatever the behavior of these plants may be under the usual cultural conditions, and the edaphic and climatic conditions of India, in these greenhouse experiments there was no evidence of the toxicity of root excretions of *A. sorghum*. The results obtained tend to make one skeptical as to the reality of toxic root excretions emanating from the root systems of living plants. The burden of proof should surely rest with those who make the claim that such toxic excretions exist.—CHARLES A. SHULL, *University of Chicago.*

NOTES

New Orleans Meeting.—The eighth annual meeting of the American Society of Plant Physiologists was held at New Orleans from December 29, 1931, to January 1, 1932. It was well attended, and was one of the most satisfactory meetings in the history of the organization. Joint meetings were held with the horticulturists, and with Section G of the A. A. A. S. The program committee had arranged the papers with some reference to the subject-matter, and large attendance was the rule. About sixty members and friends of the Society were present at the banquet. The occasion was one of delightful fellowship, and maintained the tradition that the plant physiologists have one of the most attractive social events of the meetings.

Many matters of business were considered, and some important decisions were made, which will concern the future welfare of the Society. A few of the most important matters are reported briefly in this number of PLANT PHYSIOLOGY.

Incorporation.—The Committee on Incorporation, Dr. WALTER THOMAS, *chairman;* Dr. BURTON E. LIVINGSTON, and Dr. J. B. OVERTON, reported the results of the investigation of the advantages and disadvantages of incorporation of scientific societies. Weighing the matter carefully, the committee recommended incorporation either under the laws of the state of Wisconsin, or under those of the District of Columbia. After discussion, it was voted by the Society to incorporate, preferably under the laws of the District of Columbia. This change in legal status will prove advantageous in several ways. It will no doubt make it simpler to accept and administer bequests. It will also relieve the individual members of the Society from financial responsibility for the corporation's debts, if it should have any. Moreover, it will be conducive to stability and permanence of organization. The officers of the Society, authorized to take this step, will proceed at once with the legal steps necessary to incorporation.

Editorial Assistance.—The Society voted funds for an assistant to the editor of PLANT PHYSIOLOGY. This action is very much appreciated in the editorial office. The journal has almost doubled in size since its initial volume, and the assistance provided will lighten the burden very materially. A very capable assistant has been secured, with arrangements which we hope may be permanently satisfactory. With this help it should be possible in the immediate future to begin issuing the journal during the month for which is it dated.

Secretarial Office.—Expenses of the Secretary's office tend to exceed the amount allowed under the constitution. As the Society has accumulated reserve funds which are unassigned to other purposes, it was voted to allow a limited use of interest from such unassigned funds to supplement the current income from membership fees. The largest drain on the Secretary's funds is the International Address list, which of course should be issued at about five-year intervals. Publication of the membership list might be made a biennial matter instead of annual, without very serious loss in efficiency.

Life Membership Award.—The seventh award of the Charles Reid Barnes life membership in the Society was announced at the annual dinner on January 1. The announcement was made by Dr. R. H. CARR, chairman of the committee, and the member honored is Dr. CHARLES ORVILLE APPLEMAN, of the University of Maryland.

Dr. APPLEMAN was born at Millville, Pa., December 6, 1878. He holds degrees from the Bloomsburg (Pa.) State Normal School, Dickinson College, and The University of Chicago (Ph.D., 1910). He was Professor of Biology at Lombard College, 1904–1908, and in 1910 was appointed plant physiologist in the Maryland Agricultural Experiment Station. Since 1917 he has been Professor of Plant Physiology and Biochemistry in the University of Maryland, and Dean of the Graduate School. He now heads the Department of Botany in the institution he has served so faithfully.

An incident brought out in Dr. APPLEMAN's response to the award is the fact that he was a member of the anxious group of students who waited in vain for a lecture at the time of Dr. BARNES's fatal accident. It is very fitting that the most distinguished member of that group has been added to the roll of life members who form a living memorial to BARNES, who is remembered by all who knew him, as a great teacher, a cordial friend, and a keen critic in the field of plant physiology.

Stephen Hales Prize.—The third Stephen Hales Prize is to be awarded at the ninth annual meeting of the Society at Atlantic City, New Jersey, in 1932. The committee in charge of the selection consists of the following members: Dr. A. L. BAKKE, *chairman;* Dr. D. R. HOAGLAND, and Dr. W. W. GARNER. The award is made every second year to some investigator in North America who has done work considered by the committee to be worthy of this honor. Announcement of the award is made at the annual dinner.

Summer Meeting.—A program of unusual interest is being arranged for the summer meeting of the American Society of Plant Physiologists, which is to be held at the University of Wisconsin, Madison, on July 11, 12, and 13, 1932. This meeting will occur jointly with that of the Corn Belt Section of the American Society of Agronomy. The program will be built around the environmental relations of plants from the view-point of biochemists, physiologists, agronomists, pathologists, and bacteriologists. It is proposed to confine the discussion of the first few sessions to a number of subjects of basic interest which will permit the utilization of the more significant examples of field, greenhouse, and laboratory work as illustrative material for presenting principles and technique. On the last day a field trip is being arranged to visit the outlying experimental areas in Wisconsin which represent a wide range of environmental conditions. The local representative of the Society for this meeting at the University of Wisconsin, is Dr. L. F. GRABER, of the Agronomy Department at Wisconsin.

Accommodations at Madison.—Members attending the summer meeting in July may be able to secure accommodations at the University of Wisconsin dormitories at $1.50 per day, with meals at 20 to 60 cents. The principal hotels and their rates are as follows:

	Single	Double
Loraine Hotel, 123 West Washington Ave.,	$2.00–$3.00 up,	$3.00–$5.00
New Park Hotel, 22 S. Carroll St.	1.50– 2.00	2.50– 3.00
Belmont Hotel, 31 N. Pinckney	2.00– 2.50	3.50– 4.00
Cardinal Hotel, 416 E. Wilson	1.50– 2.00	2.00– 2.50

Summer Meeting, A. A. A. S.—The summer meeting of the American Association for the Advancement of Science is to be held at Syracuse during the week beginning June 20th, 1932. These summer meetings provide most pleasant features, and the Finger Lake region is enticingly beautiful. The American Society of Plant Physiologists has made no plans to participate in this meeting, since the meeting at Madison had been arranged before the Syracuse meeting was announced. Members of the Society in the surrounding territory may find it possible to participate in a local way. Notices as to fares, accommodations, etc., may be obtained from *Science*.

Portraits.—We are greatly indebted to Dr. G. J. PEIRCE, of Stanford University, for the privilege of using the portrait of Sachs published in the January number of PLANT PHYSIOLOGY. The photographs of the Laboratory at Würzburg, one of which, with SACHS seated in his work room is published in this issue, have been secured through the efforts of Dr. F. M. ANDREWS, chairman of the Memorial Committee, and Dr. R. B. HARVEY. The frontispiece in this issue was sent to Prof. Andrews by Dr. BURGEFF, of the Botanical Institute, Würzburg.

The portraits which have been published in PLANT PHYSIOLOGY will be furnished while they last, at 10 cents each, with 15 cents additional for postage, packing and exchange. This is at the rate of $1.45 for 13, $1.55 for 14, etc. Broken lots can be ordered at the same rate provided the charge for postage, etc., is included.

Chemical Methods.—There are still a few sets of the pamphlets published by the Committee on Chemical Methods available to plant physiologists who would like to own a set for personal use. They can be obtained from Dr. W. E. TOTTINGHAM, President of the Society, at the University of Wisconsin.

Program Committee.—The Program Committee for the Atlantic City meeting has been appointed by President TOTTINGHAM, and consists of the following members: Dr. JOHN W. SHIVE, New Jersey Agricultural Experiment Station, *chairman;* Dr. GEORGE H. DUNGAN, University of Illinois; Dr. MARY E. REID, Washington, D. C.; Dr. C. O. APPLEMAN, University of Maryland; and Dr. RAYMOND KIENHOLZ, Connecticut Agricultural Experiment Station. The committee members will appreciate the cooperation of all other members in arranging for this meeting. Special SACHS programs are being planned as a part of the celebration of his centennial. The Memorial Committee is represented in the Program Committee in order to facilitate cooperation between the committees.

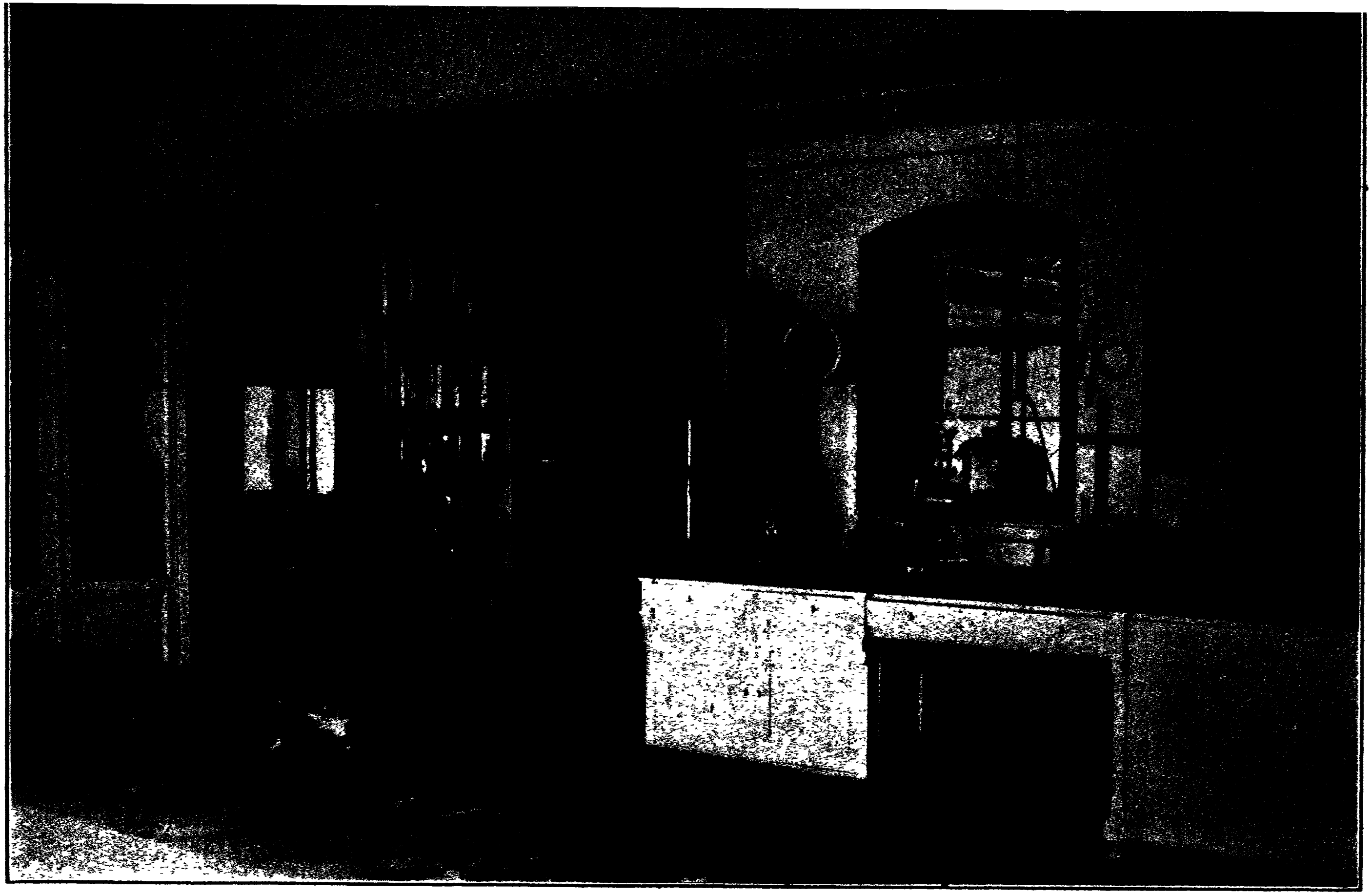
Agricultural Biochemistry.—A new work in this field has been published by John Wiley and Sons, New York. The authors are Professor R. A. DUTCHER, and Professor D. E. HALEY, of the Department of Agricultural and Biological Chemistry, Pennsylvania State College.

The material is presented in three parts. Part I is general and introductory, giving a history of the rise of agricultural chemistry, the chemistry of protoplasm, the properties and classification of the carbohydrates. lipides, and proteins. There is also a chapter on the physical state of matter, which includes the properties of solutions, nature of membranes, H-ion concentration, buffers, colloids, adsorption, etc., and a chapter on enzymes.

Part II considers the plant, germination, mineral nutrition, fertilizers and other soil factors, insecticides, and the formation and utilization of organic compounds. Part III deals with the animal foods, digestion, metabolism of tissues, protein quality, vitamins, etc.

The book is simple enough to serve as an introduction, and, in addition to covering the field, contains a summary of the valuable work done at the Pennsylvania State College on plant and animal nutrition. These distinctive features will be much appreciated by users of the work. It will be welcomed as a text suited to the student beginning his interest in biochemistry. The book is priced at $4.50 by the publishers.

Principles of Soil Microbiology.—The second edition of this splendid work by Dr. S. A. WAKSMAN, of the New Jersey Agricultural Experiment Station, has been published by the Williams and Wilkins Co., Baltimore, Maryland. It has been carefully revised, some chapters rewritten, and other chapters added which tie the work more closely to the field of plant nutrition. Some condensation and omission of material has permitted a book of about the same size as the original edition in 1927. The work has gained in value with the revision, and is the outstanding contribution in the field of soil microbiology. It is an indispensable reference work for the bacteriologist, soil scientist, and plant physiologist. The price is $10.00, the same as the first edition.



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EFFECTS OF THE PHYSICAL ENVIRONMENT ON THE PHYSICO-CHEMICAL PROPERTIES OF PLANT SAPS, AND THE RELATION OF THESE PROPERTIES TO LEAF TEMPERATURE[1]

GLENN A. GREATHOUSE

(WITH SEVEN FIGURES)

I. Introduction

The importance of studies on the physico-chemical properties of expressed plant saps, as indices of physiological and ecological relationships, has long been recognized. Such studies have been confined for the most part to the measurement of osmotic pressure, electrical conductivity, and more recently, the percentage of bound water in the expressed sap.

This investigation deals with certain physico-chemical properties of expressed plant saps as influenced by age of plant, soil moisture, and the concentration of salts in the soil. The relationship between osmotic pressure of the cell sap and leaf temperature was also studied.

II. Literature review

That physico-chemical properties of cell sap in plants are receiving much attention among scientific investigators is evidenced by the volume of literature appearing on this subject. Among the contributions are some excellent and comprehensive reviews (**20**, **35**, **41**, **45**). It will therefore be unnecessary here to do more than develop a general view-point of the problem, including only those aspects which have been touched upon in this investigation.

MEYER (**41**), GORTNER (**20**), and SAYRE and MORRIS (**53**) have given a comprehensive review of the methods employed in the extraction of plant saps.

HARRIS and co-workers (**26**, **20**) and many others have made extensive use of the method of depression of the freezing point in studies of plant distribution, development, and their relationships to the physical environment. **Korstian** (**35**), **Reed** (**51**), **Gail** (**17**), **Harris** and co-workers (**20**), and others have shown that soil moisture has an obvious effect upon cell-sap concentration. The addition of water to the soil usually diminished the concentration of the plant sap.

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[1] This investigation was begun at the University of Illinois, and continued at Duke University.

Harris and co-workers (**25, 26**), **Walter** (**55**), **Cavara** (**6**), and others have studied the effect of salt concentration in the soil upon the physicochemical properties of the plant sap. **Cavara** reported cryoscopic determinations on saps of high concentration, and in 1905 gave results in full for a considerable series of determinations. His maximum values were found in the sap of halophytes which grew in localities where the concentration of the soil solution increased with the advance of the season. He found freezing-point depressions of 7.25° to 8.50° in *Halocnemum strobilaceum,* and 7.48° in *Salicornia fruticosa.* His determinations were made on sap extracted without previous treatments of freezing, which is necessary to render the tissues permeable, and which has been shown to be essential by **Dixon** and **Atkins** (**9**) and others. **Atkins** has already pointed out that these are probably submaximum, because of incomplete extraction.

In 1920, **Harris** and co-workers found in an alkaline area in Utah a plant, *Atriplex nuttallii,* the sap of which had a depression of the freezing-point of 14.4° C., which is equivalent to an osmotic pressure of approximately 173 atmospheres. According to **Gortner** (**20**), **Harris** observed a slightly greater depression than this in the sap of *A. confertifolia.* It is obvious that the salt concentration of soil in which plants naturally grow is of great physiological and ecological importance.

A number of investigators have shown that the concentration of tissue fluids of a given species is not constant at all times, and that there exists a gradient within the shoot of an individual plant. Probably the first to give exact data on this phenomenon were **Dixon** and **Atkins** (**9**). While working with untreated leaves of *Fraxinus excelsior, Vitis veitchii,* and *Magnolia acuminata,* they found a slightly greater depression of the freezing-point for sap from the more distantly placed leaves. However, the osmotic pressure of the sap from leaves at the same level, but at different times and under different conditions, did not correspond by any means; yet it was usually higher than that of the leaves at a lower level. **Harris** and co-workers (**24**) used the method of pre-freezing in ice-salt mixture. Their results on the depression of the freezing point are similar to those of **Dixon** and **Atkins**, but they found that the relative concentration of electrolytes decreased from the lower to the higher levels, which they believe is due to increased photosynthesis in the upper regions of the tree rather than to the concentration of salts from the soil solution by increased transpiration.

Other workers have observed higher concentrations in the sap of leaves or stems near the apex. **Reed** (**51**) found that the concentration of the sap in the apical portion of a stem was greater than that in the basal region. His data show a greater tendency toward fluctuation in the stem than in the leaf. **Hurd-Karrer** (**30**) states that the specific gravity of the sap extracted from the internodes of corn stalks was higher toward the top of the plant. **Fernald** (**15**), working with *Philadelphus* and privet, noted irregularities for the cell-sap concentration of leaves toward the tip of branches. In two cases a marked drop in the sap concentration of the uppermost leaves was shown. **Korstian** (**35**) reported the sap concentrations to be higher for leaves which were at greater distances from the water supply. **Pringsheim** (**50**) observed in succulents that the concentration was greater in the upper leaves. He accounted for the internal translocation of water from lower to upper leaves during wilting by the difference in osmotic pressure. **Beck** (**3**) recently used the same method as employed by **Pringsheim**, and states, "even though it seems evident that the water passes from the older parts to the younger parts, **Pringsheim**'s explanation is not at all convincing. He did not have a clear conception of the relation between the direction of flow and the osmotic quantities of the cells. His explanation was based on the assumption that the water must flow in the direction of the O_g gradient, which is not true. The gradient itself which he claimed to exist, is doubtful (**9**, p. 102). (It should be remembered in this connection that **Pringsheim** employed KNO_3 as plasmolyzing agent, which is unreliable, *cf.* **3**, p. 431.") **Zalenski** (**57**), dealing with other peculiarities of the upper leaves of shoots, also pointed out differences of osmotic pressure. **Iljin** and **Saboleva** (**32**) reported similar findings, and in addition found a greater osmotic value in the distal portions of the leaf blade as compared with the basal portions. It must be borne in mind that the last three investigators used the plasmolytic method of determining the cell-sap concentrations. **Eaton** (**12**), working with cotton leaves, reported that the freezing-point depression of the extracted sap from the leaves decreased from 1.57° C. for leaves 74 days old to 1.10° for leaves which were 10 days old. Finally, **Martin**, **Harris**, and **Jones** (**39**) found the freezing-point depression to be greatest in the juices of the terminal leaf of sorghums, with a progressive decrease in the leaf juices from the top of the plant downward. Also the freezing-point depression of the juices of different internodes was about the same in the flowering stage but increased from the top down to the fourth internode when the plant approached maturity.

The earlier literature in regard to the temperature of leaves has been thoroughly reviewed by **Ehlers** (**14**), and will be mentioned in this paper only in the discussion of the various phases of the experiments. **Miller** and **Saunders** (**42**) and **Eaton** (**12**) reported that young leaves were cooler than old leaves, and that the temperature was higher for wilted than for turgid leaves. These relationships are in general keeping with the observations of KIESSELBACH (**33**), LOFTFIELD (**38**), and CLUM (**8**), who used various methods to arrive at their comparisons.

An examination of the literature dealing with the transpiration rate of leaves of different ages shows that investigators, using a number of methods, have found great variability in the directions which the gradient takes in different plants.

ALEXANDROV (**1**), working with the sunflower, found that the upper leaves transpire much more vigorously than the lower ones. ZALENSKI (**58**) carried out experiments on the transpiration of different leaves on the same plant and drew conclusions similar to those of ALEXANDROV. SCHECHNER (BURGERSTEIN **5**) reported that the transpiration of young leaves of many plants was greater than that of older leaves, and that in some other plants the leaves of intermediate age had lower transpiration rates than either older or younger leaves. On the contrary, OVERTON (**46**), while working with *Cyperus papyrus,* found that the older leaves transpire more vigorously than the younger ones. SEELIGER (BURGERSTEIN **5**), working with cut leaves from woody plants, and FLEISCHMANN and HIRZEL (BURGERSTEIN **5**), studying the leaves of hop, drew conclusions very similar to those of OVERTON. GIDDINGS (**19**) found that both the higher and lower leaves of *Silphium laciniatum* transpired less rapidly than did the leaves borne near the middle of the plant. KOKETSU (**34**) reported that the stomatal transpiring power of *Coleus* leaves was greatest at the height of their development. He used the hygrometric paper method for his studies. PALLADIN (**47**) states that the transpiring rate is highest during the period of the greatest activity of the leaf, that is, while it is still growing. He assumes this to be due to both cuticular and stomatal transpiration. Recently EATON (**12**) found that young leaves of cotton were cooler than old leaves. The temperature of the leaves 82 days old was 2.3° C. below that of the air, whereas the temperature of the leaves 26 and 20 days old was 4.1° below that of the air. He found that the temperature of very young leaves, 3 days old, was approximately the same as that of leaves 82 days old.

III. Methods and apparatus

The plants used in this investigation were: five varieties of soy beans (*Soja max*), Illini, Manchu, Ebony, Harbinsoy, and Mandarin, respectively; sunflower (*Helianthus annuus*), milo (*Holcus sorghum* var. *durra*), and cabbage (*Brassica oleracea* var. *capitate,* four varieties). The soy beans were secured from the Department of Plant Breeding at the University of Illinois. The seeds were inoculated by means of soy bean nodules, secured from a field in which this plant had formerly grown.

These plants were selected for various reasons: (1) All of them, with the exception of cabbage, have a rather shallow and limited root system, which probably renders them sensitive to variations in moisture conditions. (2) All of them are commonly grown as annuals in various states. The sunflower is recognized as being genetically a mixture, but data presented later will show that the results are not greatly variable even for such a plant.

The plants were grown in the greenhouse under different moisture conditions and soil salt concentrations. The experiments were performed upon normal plants grown in pots, 4 to 8 inches in diameter. The soil used in the containers was a sandy loam in good tilth, having a field-carrying capacity of 35.10, a moisture equivalent of 9.71, and a wilting coefficient of 4.86. A sufficient quantity of soil was prepared at the beginning of the experiments so as to use but one type of soil and to use it only once. The soil was sterilized and thoroughly mixed before it was used. Soil samples were taken, by means of a special cork-borer, from time to time from the various pots, to determine an average soil moisture throughout the growth of the plants. The water content was then determined. These water-content values are expressed as percentages of the dry weight of the soil. The soil in the containers was kept at a moisture content of approximately the given percentage desired, by the frequent addition of water to replace that lost by evaporation. Frequent additions of water were made by the use of a series of auto-irrigators, designed by Professor CHAS. F. HOTTES, but modified to adapt it to this investigation. Neither the auto-irrigator nor the modifications mentioned will be described here since Professor HOTTES contemplates doing further work with it before publication of his results. The field-carrying capacity, the moisture equivalent, and wilting coefficient were determined by the method described by BRIGGS and SHANTZ (4).

The leaf and stem material used was collected from the respective parts of the plant, and placed as rapidly as picked into large test-tubes, 1.75 inches in diameter and 7 inches in length. Tubes of this size accommodate from 50 to 60 gm. of plant parts. Each leaf sample was then weighed, and in all cases, except where different methods of treating the tissue before applying pressure were employed, the tubes containing the plant parts were plunged at once into a freezing box of "dry ice." The freezing box with the samples was set in a refrigerator for the period of freezing, which was always 10–12 hours. Leaves and stems treated in this manner are still frozen when the tubes are unstoppered for extraction of the tissue fluids. All leaf samples were collected between 3:30 and 4:00 o'clock in the afternoon, except where otherwise stated, and determinations were made upon each set within one to two hours after they had been extracted.

Different methods of treating the tissue, before applying pressure, were tried out. Pre-freezing with dry ice proved to be the most satisfactory for this investigation. The amount of sap obtained from a sample depends upon its size, the age and kind of tissue, water content, and on its treatment.

A small but powerful hydraulic press, equipped with a test cylinder 1.12 inches in diameter, with necessary filters and accessories, was used for obtaining the sap samples. This is the standard press, LPI, with 1.12-inch diameter test cylinder and filter pads and pan (S–1722), sold by Fred S. Carver, New York. The pressure was sufficient to leave the press cake relatively dry.

A uniform time of draining and a uniform pressure were used. The following procedure served as a standard: a 50-gm. sample of tissue was subjected to 5000 pounds per square inch, drained for four minutes after maximum pressure was reached, and the volume of sap extracted was measured. These samples were tightly stoppered and placed in ice-water pending determination of the freezing point.

ESTIMATION OF DRY SUBSTANCE IN PLANT SAP BY REFRACTOMETER

The water content of the tissue was obtained from a duplicate sap sample and the water left in the press cake. The dry substance content of the sap was obtained by the refractometer method of GORTNER and HOFFMAN (**21**). An Abbé refractometer (Zeiss) was used.[2] Instead of using the sugar scale for the dry-matter reading, the dry substance was obtained from GEERLIG's table used in determining the dry substance in sugar house products (**2**). The percentage of water in the sap was obtained from published tables of the United States Bureau of Standards (**49**). This method was checked by drying a known quantity of sap in the oven at a temperature of 65°–70° C. for a period of 196 hours.

DETERMINATION OF OSMOTIC PRESSURE OF EXTRACTED LEAF SAPS

The freezing point of the sap was determined in the usual freezing-point apparatus with the use of a Beckmann thermometer certified by the Bureau of Standards. At least two determinations were made upon each sample, and the average of closely agreeing duplicates was taken as the freezing point. The observed freezing-point depressions were corrected for undercooling by the table of HARRIS (**22**). The corrected freezing-point depression readings were converted into equivalent osmotic values from HARRIS and GORTNER's tables (**23**).

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[2] Lent for the investigation by courtesy of the Department of Chemistry of Duke University.

DETERMINATION OF BOUND WATER

The method employed in this investigation was essentially that introduced by NEWTON and GORTNER (**43**) but with slight modifications in order to improve the accuracy of the technique. The procedure, as modified, is as follows: The dry substance content is obtained by a refractometer. Then the freezing-point depression of the fresh juice is obtained by the BECKMANN method, making the necessary correction for undercooling. Next a fresh sample of juice containing exactly 20 gm. of water is weighed out into a 100-cc. Erlenmeyer flask; this is a convenient quantity for a duplicate determination. Since the principle of the method depends upon the determination of small differences in the amount of free water in the juice, it is important that the juice be accurately weighed. The weight of juice required to contain exactly 20 gm. of water can readily be calculated, after knowing the dry substance content as indicated by the refractometer. To the accurately weighed portion of juice are added 6.8448 gm. of finely pulverized sucrose; this is just sufficient quantity to make a molar concentration in the total water percentage. The sucrose samples were previously weighed out into small porcelain dishes and stored in a desiccator until required for use. After the sucrose has been added to the flask containing the juice it is stoppered and placed in a bath of ice slush and gently moved back and forth in a horizontal motion. Care is used to avoid frothing, which would probably bring about physical changes in the colloids. The time required for the sucrose to dissolve in the juice was found to be 20 to 25 minutes, when the sucrose was finely pulverized.

After the addition and solution of the sucrose, the freezing-point depression of the juice sample is redetermined. This value is usually found to be greater than the sum of the original freezing-point depression of the juice and the sugar solution determined separately. This excess depression is considered to be due to the fact that part of the water is being held as imbibed water by the hydrophilic colloids in the juice and is thus unavailable as a solvent. The molecular concentration of the sugar will therefore be increased more than the theoretical amount; hence an excess depression of the freezing point. The magnitude of the excess depression is taken as a measure of the water held in such a way as not to be available for the solution of the sucrose. The percentage of "bound water" is in turn taken as an index of the relative colloidal content of the plant juice.

The possible sources of error in this method have been discussed by its originators. However, there are certain precautions to keep in mind. The same portion of juice should never be frozen twice, since the frost precipitation of proteins alters the hydration and consequently invalidates the results. For this reason, when bound-water studies were being made, the plant tissues used were finely ground in a food chopper rather than prefrozen. It is likewise necessary to keep dry the neck and upper wall of the flask. Again, the validity of the method as a measure of the hydrophilic colloid content of a sap rests upon the assumption that there are no other substances present in the juice which bind appreciable amounts of water. Probably the one non-colloidal substance which is likely to be present in plant juices in sufficient quantity to bind appreciable amounts of water is sucrose. This fact should be borne in mind when interpreting the results of an investigation upon different plant saps and the same plant sap when collected under different environmental conditions.

The percentage of bound water is calculated as follows (symbols and formula taken from NEWTON and MARTIN, **45**):

Δ = freezing-point depression of the plant juice.
Δa = freezing-point depression of the juice after a molar concentration has been added.
$\Delta s = \Delta a - \Delta$, the additional depression due to the added sugar.
Δx = Δs minus the freezing-point depression (determined experimentally) of a molar solution of the sugar used.

$$\text{Bound water} = \frac{\Delta x \cdot 89.2}{\Delta s},$$

where 89.2 is the percentage of free water in a weight molecular solution of sucrose, assuming the formation of sucrose hexahydrate, as explained by NEWTON and GORTNER (**43**).

LEAF TEMPERATURE MEASUREMENTS

No procedure for the direct determination of the individual transpiration rates of attached leaves under experimental conditions has yet been devised, except for the method employed by ALEXANDROV (**1**). His method consisted of wrapping and unwrapping, in turn, the leaves of the upper and lower zones respectively; thus he was able to compare the intensity of transpiration of the two sets of leaves without detaching any of them, and at the same time to employ that most satisfactory of all methods, the weighing of intact, rooted plants. The thermo-electric method was used in this investigation to obtain the leaf temperature. The leaf-temperature method is not only convenient and applicable to attached leaves, but it has a further advantage in that it is rapid. The leaves until the moment of a measurement are fully exposed to a standard light, and during the few seconds required for the reading there is little opportunity for a material change in leaf temperature. Several investigators have found a high correlation between transpiration rates and leaf temperatures; therefore it is possible to use this method to secure the measurements and comparisons.

The thermo-electric method described by EATON and BELDEN (13) is generally followed. The wall galvanometer[3] used was provided with a telescope and scale, and was formerly listed by Leeds and Northrup as no. 2239–d, type P. When purchased it had a coil resistance of 2000 ohms, a sensitivity of 0.002 microcoulomb per millimeter on the scale, and a period of 26 seconds. As wired for use, it had a sensitivity of 0.1204° C. per millimeter. The thermocouples were of no. 36 copper and constantan wire with soldered junctions. They were inclosed in a piece of rubber tubing and attached to a wooden handle as described by EATON and BELDEN.

The galvanometer and thermocouples were standardized by passing a known current through them and by immersing the respective junctions in a series of water baths of different temperatures. In preparation for taking the readings, the scale zero was brought to the cross hair, while the air junction was protected from the light by its paper cone and the leaf junction by the shadow of another piece of paper.

For a short time the thermo-electric apparatus was placed in the greenhouse near the experimental plants; but because relatively large fluctuations in direct sunlight were found, especially when the temperature was high and when the air was in motion even during a brief period of a few seconds, the apparatus was removed to a room where the temperature, humidity, and air currents were nearly constant, at least over a period of several seconds and even minutes. A standard light (standard candle power 61.893) at a distance of three feet was used under these conditions. Most of the temperature determinations were made by two persons. One clasped the leaf surfaces over the thermo-junction while the other recorded the swing of the galvanometer when it came to rest. This procedure permitted fairly rapid work.

Six potted plants, growing under uniform conditions of moisture, etc., were selected at random for the leaf-temperature studies. Three temperature measurements were made on each of the leaves of six plants. Beginning with the lowest leaves, which did not show any signs of deterioration, the temperature of each leaf was measured, proceeding from the older to the younger leaves in turn, while the plant was turned whenever it was necessary to get equal exposure to the light. As soon as the leaf-temperature measurements were completed, these leaves, with similar ones from other plants grown under like conditions, were gathered for the determination of the tissue fluids. The preceding method provided 36 readings for each temperature mean. The mean temperature of the leaves in each of these groups was averaged for the comparison. The probable errors of the means were calculated by the usual formula, since all the determinations exceeded twenty in number. The leaf temperatures were made on leaves, numbers 60–72 and in some cases 72–84, but owing to the small size of leaves numbering 72–84 it was deemed not feasible to collect them for sap analyses.

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[3] Lent for this investigation by courtesy of the Physics Department of Duke University.

IV. Experimental data and discussion

EFFECT OF DIFFERENT METHODS OF TREATING TISSUE PREVIOUS TO EXTRACTION

A comparative study was made of several methods of treating leaf tissues, to determine the effect upon the volume of sap which could be extracted from them under pressure, and the osmotic concentration of this extracted juice. Parallel series of determinations were made on leaves of plants grown in soil containing a very low supply of available water and on others grown with approximately the optimum supply of soil moisture. The leaves were equally divided into four lots and received the following treatments. The leaves of lot no. 1 were placed under a pressure of 5000 pounds per sq. in. without being given any previous treatment; those of lot no. 2 were pre-frozen with solid CO_2; those of lot no. 3 were pre-frozen with an ice-salt mixture; and those of lot no. 4 were previously ground into shredded pulp by means of a food grinder, before extraction of sap by means of pressure.

TABLE I

EFFECT OF DIFFERENT TREATMENTS OF SOY BEAN LEAVES UPON EXTRACTABLE AMOUNT OF SAP AND UPON OSMOTIC CONCENTRATION OF EXTRACTED SAP

PRESSURE, 5000 POUNDS PER SQ. INCH; TIME OF DRAINING, 4 MINUTES

DATE (1930 OCT.)	METHOD OF TREATMENT	MOISTURE CONTENT OF TISSUE	VOL. OF SAP PER 100 GM. TISSUE	MEAN AGE OF LEAVES	TOTAL WATER EXTRACTED	OSMOTIC PRESSURE IN ATMOSPHERES	SOIL MOISTURE (DRY WEIGHT)
		per cent.	*cc.*	*days*	*per cent.*		*per cent.*
28	Solid CO_2	(85.3)*	(60.3)	(24)	(70.6)	(9.178)	(17.0)
31	Solid CO_2	79.8	53.6	25	67.2	10.079	9.0
28	Ice-salt mixture	(85.3)	(53.4)	(24)	(62.6)	(9.105)	(17.0)
31	Ice-salt mixture	79.8	37.5	25	47.5	9.154	9.0
28	Ground	(85.3)	(52.2)	(24)	(61.2)	(9.045)	(17.0)
31	Ground	79.8	41.3	25	51.9	10.156	9.0
28	Untreated	(85.3)	(31.6)	(24)	(37.1)	(7.361)	(17.0)

* Brackets indicate that plants were grown in soil containing approximately optimum soil moisture.

Table I records the results of a representative series of determinations upon the leaves of soy bean, variety Manchu. The water content of the leaves was 5.5 per cent. higher in the plants grown under the more favorable water supply, a fact which may be expected to have some influence on the volume of tissue fluids which could be extracted. For plants grown under more favorable conditions of soil moisture, approximately the same volume of tissue fluid was extracted from samples frozen with solid carbon dioxide, from samples frozen with ice-salt mixture, and from samples ground into pulp by means of a food grinder. Whereas, for plants grown under less favorable conditions of soil moisture, only with the sample frozen in solid carbon dioxide was a volume of sap secured which compared with the volumes expressed from plants grown under more favorable conditions of soil moisture. The untreated sample gave a smaller volume of plant sap than any of the treated samples.

MEYER's (**41**) work with pine leaves collected during the summer and winter months shows that pre-freezing with solid CO_2 yields the greater volume of sap. Thus his results are substantiated by those of table I.

COMPARISON OF REFRACTOMETER AND OVEN METHOD FOR DETERMINING DRY SUBSTANCES IN PLANT SAPS

In this type of investigation a direct and yet rapid method of determining the content of dry substance in biological fluids must replace the usual method in which materials are desiccated in an oven. GORTNER and HOFFMAN (**21**) proposed the use of the refractometer for this purpose, for the reason that it is more economical of juice, time, and apparatus. It was deemed necessary, however, to compare these two methods to determine the degree of accuracy of the more rapid method.

The determinations on oven-drying were carried out by desiccating 10-gm. samples of juice in porcelain dishes of 4-cm. diameter, in duplicate, in an oven at 65–70° C. for 196 hours. This excessive length of desiccation seemed necessary because of the difficulty with which the last traces of moisture are driven from the gummy residues left on evaporation. Undoubtedly, volatile materials other than water are given off by oven-drying; however, these substances are thought to constitute a very small portion of the dissolved substances and therefore do not introduce a serious error.

The results in table II show that refractometer readings give only 89.75 per cent. of solids found by oven-drying. Therefore a correction of 10.25 per cent. was used in all data for the dry substance when determined by the refractometer method. The data in table II confirm in general those of NEWTON and MARTIN (**45**), which were published just after the writer had concluded his experiments. However, they found it necessary to make a larger correction than these data show. This difference may

TABLE II

DRY SUBSTANCE IN EXTRACTED JUICE AS DETERMINED BY REFRACTOMETER AND OVEN

SPECIES	DATE OF COLLECTION (1930)	DRY SUBSTANCE		PERCENTAGE OF REFRACTOMETRIC READING FOUND BY OVEN-DRYING
		BY OVEN DRYING	BY REFRACTOMETER	
		per cent.	*per cent.*	*per cent.*
Soja max var. mammoth yellow*	Sept. 15	15.45	17.14	90.1
Soja max var. Virginia*	" "	13.99	15.36	91.1
" " " " * + 5% sucrose	" "	18.69	20.73	90.2
Helianthus annuus*	" "	11.66	13.06	89.2
Zea mays*	" "	11.41	12.87	88.7
Soja max var. Manchu†	Oct. 14	8.97	10.09	88.8
Soja max var. Illini†	" "	9.27	10.23	90.6
" " " " †	" 22	9.267	10.41	89.02
Soja max var. Manchu†	" "	8.36	9.28	90.0
Average				89.75

* These plants were collected from fields about 3 miles northwest of Durham, N. C.
† These plants were grown in the greenhouse.

be due to the fact that they used a vacuum oven at a temperature of 96°–97° C. for the last 48 hours of desiccation, thereby making it more probable that the last traces of the moisture were driven from the residue. If the correction used in this investigation is too low, the bound-water determinations given later in this paper are in consequence slightly low.

A deviation of 0.2–0.4 per cent. represents the probable error in dry substance shown in the data in table II. This is not of sufficient magnitude to affect the comparative values.

EFFECT OF STORING PLANT SAPS AT 0° AND 20° C.

The tissue fluids were stored for a short time only after being extracted, before the desired determinations were made. The interval between extraction and the making of the determinations seldom exceeded one hour. In this interval the changes in the properties of the tissue fluids should be inappreciable, when stored at 0° C.

In order to ascertain the effect of time and temperature upon the osmotic value, samples of juice were divided and one part was stored at a constant temperature of 0° and the other portion at 20° C. The samples were held for a maximum period of about 6 hours. At desired intervals portions were withdrawn for the determination of the osmotic value. The results of this experiment are shown in table III.

TABLE III

EFFECT OF DURATION AND TEMPERATURE OF STORAGE ON THE OSMOTIC VALUE OF PLANT JUICES

PLANT JUICE USED	DATE (1930)	TIME OF STORAGE	TEMP. OF STORAGE	DEPRESSION OF FREEZING POINT	OSMOTIC PRESSURE
		hours	°*C.*	°*C.*	*atm.*
Soja max var. Manchu, 8.5 per cent. solids	September 30	0.0	0	0.783	9.43
		1.0	0	0.784	9.44
		2.3	0	0.803	9.67
		4.2	0	0.811	9.76
		6.0	0	0.819	9.86
		0.50	20	0.820	9.87
		1.45	20	0.885	10.66
		2.80	20	0.908	10.94
		3.50	20	0.923	11.02
		5.23	20	0.946	11.39
Soja max var. Illini, 9.1 per cent. solids	October 2	0.00	0	0.776	9.35
		1.10	0	0.781	9.41
		2.45	0	0.793	9.55
		4.30	0	0.807	9.62
		6.10	0	0.806	9.61
		1.45	20	0.821	9.89
		3.00	20	0.843	10.16
		4.68	20	0.870	10.48
		6.70	20	0.884	10.65

These results show that the osmotic pressure in plant saps in most cases increased gradually during storage, the greater change occurring at 20° C. This increase in osmotic pressure is to be expected owing probably to hydrolysis of various substances in the juice. The rate of change in osmotic pressure at 0° C. is so slight during the first hour, the time normally required for the routine determinations, that the changes are considered inappreciable.

EFFECT OF MATURITY OF PLANTS ON CONCENTRATION AND OSMOTIC PRESSURE OF JUICES

It was desired to ascertain the effect of maturity of the plant upon the concentration, osmotic pressure, and percentage of moisture within the leaves of two varieties of soy beans and the sunflower.

The percentage of composition at different periods from near the flowering stage to near maturity for *Soja max,* varieties Mandarin and Harbinsoy,

TABLE IV

PERCENTAGE COMPOSITION OF LEAVES AT DIFFERENT PERIODS FROM NEAR FLOWERING STAGE TO APPROACH OF MATURITY

SPECIES	DATE (1930)	AGE OF PLANT	WATER CONTENT OF LEAVES	CONCENTRATION BY REFRACTION	OSMOTIC PRESSURE	REMARKS
		days	*per cent.*	*per cent.*	*atm.*	
Soja max var. Harbinsoy	Jan. 13	35	85.5	7.7	8.583	Soil moisture 17.0 per cent.; few flowers
	Jan. 23	45	79.2	8.9	9.065	Flowers and few pods
	Feb. 4	57	77.5	10.1	10.157	Pods about 2″ long
	Feb. 16	69	75.2	10.8	10.873	Beans in pods of medium size
Soja max. var. Mandarin	Jan. 13	36	81.8	8.6	9.046	Soil moisture 14 per per cent.; flowers and small pods
	Jan. 23	46	78.3	10.4	10.123	Pods 1–1½″ long
	Jan. 28	51	74.4	11.3	10.877	Pods 2″ long; small beans
	Feb. 4	58	75.5	11.0	10.912	Beans of medium size
Helianthus annuus	Jan. 15	35	91.8	5.7	7.631	Soil moisture 17.0 per cent.; no flower buds
	Jan. 28	50	86.2	7.3	8.690	Flower buds
	Feb. 7	60	84.6	8.5	8.946	Flower buds open

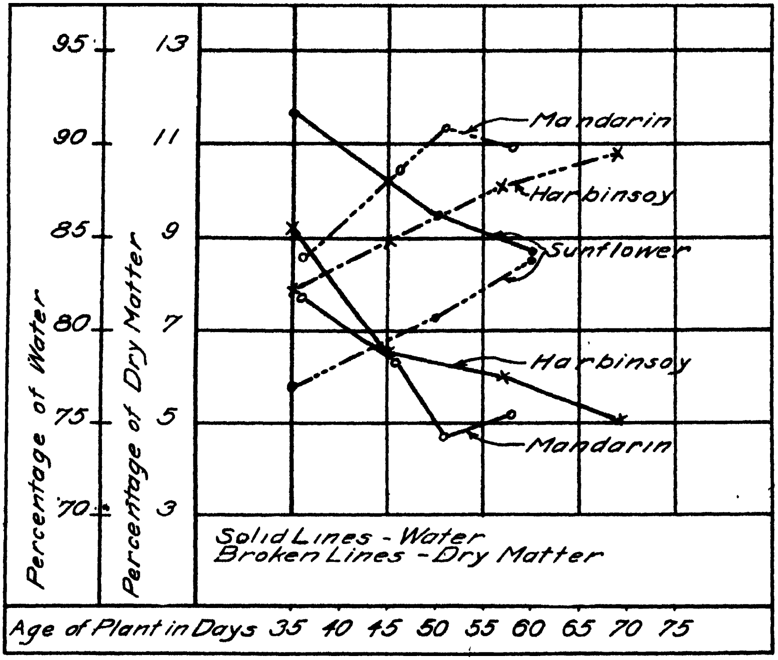

FIG. 1. Percentage of dry matter and water in soy bean and sunflower plants at different periods from near the flowering stage to approach of maturity.

and *Helianthus annuus* is given in table IV and presented graphically in figure 1.

The results in table IV show that with the approach of maturity the moisture content of the leaf tissue decreases rather rapidly, causing corresponding increase in concentration and osmotic pressure of the sap. These data are presented graphically in figure 1. The curves of this figure are of the same general trend; however, in the variety Mandarin the moisture content drops rapidly and the dry substance ascends rapidly. This is probably due to a difference in maturity of the two varieties, since the Mandarin variety bore small pods whereas the Harbinsoy had only flower buds. The sunflower plants had not reached maturity, as can be noted from the table and graph.

Welton, Morris, and Hartzler (**56**) state that the percentage of residual dry matter increased from the tasseling stage to maturity in both varieties of corn studied. The percentage of moisture decreased in the Clarage variety in the early part of the season, but the trend was reversed at maturity; the percentage of moisture decreased in the Burr Leaming throughout the period studied.

EFFECT OF COLLECTING PLANT PARTS AT DIFFERENT TIMES

On account of the well known variation in the osmotic pressure of plant saps throughout the day, collections were usually made at 4:00 P. M. However, since this investigation in part deals with the gradient of osmotic value of leaves, their water content and temperature, it was deemed necessary to make a few determinations at another hour of the day. Likewise, **EATON** (**12**) has recently drawn more or less definite conclusions on the gradient within the plant on data secured from one experiment on cotton leaves.

TABLE V

AGES, WATER CONTENT, AND FREEZING-POINT DEPRESSIONS OF EXTRACTED TISSUE FLUIDS OF LEAVES AT SUCCESSIVELY HIGHER NODES ON MAIN STEM OF SOY BEAN VAR. MANCHU; NOVEMBER 6, 1930

TIME	MOISTURE CONTENT	VOL. OF SAP PER 100 GM. OF LEAVES*	MEAN AGE OF LEAVES	FREEZING-PT. DEPRESSION	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*cc.*	*days*	(Δ)	*atm.*	*per cent.*
10: 30 A. M.	84.05	54.98	38	0.553	6.663	65.4
	84.0	57.96	33	0.608	7.325	69.0
	83.8	57.4	26	0.631	7.602	68.4
	81.9	61.3	18	0.662	7.975	74.8
	82.01	55.8	13	0.776	9.346	68.03
	Lowest ⅓ of stem			0.631	7.602	
	Middle " " "			0.658	7.962	
	Upper " " "			0.738	8.889	
4: 00 P. M.	82.5		38	0.741	8.925	
	82.4		33	0.748	8.999	
	81.9		26	0.667	8.035	
	80.7		18	0.664	7.999	
	80.6		13	0.642	7.734	
	Lowest ⅓ of stem			0.776	9.346	
	Middle " " "			0.737	8.877	
	Upper " " "			0.673	8.107	

* By doubling volumes obtained experimentally from 50-gm. sample.

The plants used for the data in table V were grown at a constant soil moisture of 15.6 per cent. The data show that if collections are made about 10: 30 A. M., results will be secured which differ considerably from those obtained at 4:00 P. M. The osmotic concentration for the successively higher nodes on the main stem are just the reverse, and the segments of the stem show the same general trend as that of the leaves.

HARRIS, GORTNER, and **LAWRENCE** (**24**), in a study of the relation between the osmotic concentration of leaf sap and the height of leaf insertion in trees, noted that the osmotic pressure increased with the height at which the leaves were collected, whereas the specific electrical conductivity decreased from the lower to the higher levels. According to these data, evidently there is an increased photosynthesis in those leaves in the upper portion of a tree, as indicated by a decreased ratio of $\frac{K}{\Delta}$ and an increased osmotic pressure. This being the case, one would expect results similar to those in table V, on account of the ratio of photosynthesis to translocation of the food materials during the period extending from late afternoon throughout the night. Whether this increase in photosynthesis in the upper and younger leaves is due to more favorable light conditions or to a more favorable ratio of the electrolytes present in the cell sap can be determined only by more extensive studies.

TEMPERATURE FLUCTUATION IN EXPERIMENTAL ROOM

The degree of fluctuation of temperature within the room in which the thermo-electrical apparatus was located was observed for a period of six hours; during this period the temperature fluctuated to the extent of ± 0.2° C. Since the variability in temperature in this room was found to be slight and did not change at a very rapid rate, it is not thought to introduce appreciable error into the leaf-temperature determinations, to be presented later. Nevertheless an average of a number of consecutive determinations was made in order that a more exact index of the temperature changes of leaves and of the surrounding air might be obtained. Each temperature mean is the average of from 20 to 36 determinations taken during a 20- to 30-minute period. It must be borne in mind, in making leaf-temperature determinations, that they are relative only to the conditions that prevail when the determinations are made. This is due to the fact that the temperature of a leaf is influenced by such factors as temperature of the air, available soil moisture, air currents, age and type of leaf, and intensity of the light exposure.

GRADIENT WITHIN THE PLANT

The leaf temperature measurements were made and the leaves collected for extraction of the tissue fluids on October 30, 1930, for the data in table VI. The temperature measurements were started at 2:00 and ended at 2:39 P. M. The leaves were collected and frozen at 4:00 P. M. The air temperature at 2:00 was 74° F. and the wet bulb was 64° F.; thus the relative humidity was 58 per cent. and the dew point 64° F. The average soil temperature at the same time was 68° F.

The leaves are grouped according to both age and position on the plant. The ages designated are the means of the leaves in each group. All of the plants used were of about the same height, and the leaves rarely differed in age from the mean more than two days.

TABLE VI

SUMMARY OF PHYSICAL PROPERTIES OF SOY BEAN LEAVES, VAR. MANCHU, AND LEAF TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 30, 1930
(SOIL MOISTURE 15.0 PER CENT.; WEATHER CLEAR AND SUNNY)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	° *C.*	° *C.*	*atm.*	*per cent.*
1–24	85.64	30	56.4	– 0.42 ± .04	0.964	11.608	66.1
24–36	85.76	25	67.1	– 0.68 ± .06	0.762	9.178	78.2
36–48	86.60	18	68.6	– 0.72 ± .05	0.756	9.105	79.7
48–60	83.60	10	67.7	– 0.97 ± .08	0.725	8.732	80.4
60–72	81.24	4		– 0.78 ± .07	..		.

* By doubling volume obtained experimentally from 50-gm. sample.

Table VII records the results obtained from leaves of variety Manchu, 38 days old, grown at a soil moisture of 9.5 per cent. The leaf-temperature measurements were started at 2:30 and ended at 3:12 P. M. The air temperature at 2:30 P. M. was 72° F. and the wet bulb was 59° F.; thus the relative humidity was 65 per cent. These plants were exposed to sunshine during the morning but it was cloudy during the afternoon.

Table VIII presents data on *Helianthus annuus* grown at a soil moisture of 15 per cent. The leaf-temperature measurements were carried out be-

TABLE VII

SUMMARY OF PHYSICAL PROPERTIES OF SOY BEAN LEAVES, VAR. MANCHU, AND LEAF TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 31, 1930
(COLLECTED AT 4:00 P. M.)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	° *C.*	° *C.*	*atm.*	*per cent.*
1–24	81.3	30	43.0	+ 1.03 ± .03	0.837	10.079	52.9
24–36	79.8	26	53.6	+ 0.91 ± .05	0.828	9.971	67.1
36–48	79.4	17	59.7	+ 0.86 ± .03	0.789	9.502	75.1
48–60	78.1	10	59.1	+ 0.64 ± .04	0.778	9.370	75.6
60–72	79.7	4	. ...	+ 0.68 ± .02	. ...	. .	.

* By doubling volume obtained experimentally from 50-gm. sample.

tween 3 : 00 and 3 : 40 P. M. The relative humidity in the experimental room was 60 per cent. The soil temperature was 69° F. The leaves were collected for freezing and storage at 4 : 00 P. M.

TABLE VIII

SUMMARY OF THE PHYSICAL PROPERTIES OF SUNFLOWER LEAVES AND TISSUE FLUIDS FOR PLANTS STUDIED ON OCTOBER 31, 1930
(CLEAR AND SUNSHINY DAY)

LEAF NOS.	MOISTURE CONTENT	MEAN AGE OF LEAVES	VOLUME OF SAP PER 100 GM. LEAVES*	DIFFERENCE BETWEEN LEAF AND AIR TEMPERATURE	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
	per cent.	*days*	*cc.*	*° C.*	*° C.*	*atm.*	*per cent.*
1–24	90.0	28	61.3	−0.81 + .05	0.754	9.081	68.8
24–36	91.3	20	64.1	−0.89 − .03	0.725	8.732	70.2
36–48	86.4	14	62.0	−0.97 − .02	0.724	8.720	71.7
48–60	87.7	10	64.0	−0.96 − .04	0.726	8.744	72.9
60–72	85.0	6		−0.93 ± .03			

* By doubling volume obtained experimentally from 50-gm. sample.

The results in tables VI, VII, and VIII are shown graphically in figures 2, 3, and 4 respectively.

The leaves were found to be progressively cooler from the older, upward, toward the younger, until leaves a few days old were reached. This was observed to be true whether the leaf-temperature determinations were made at 10 : 30 A. M. or at 4 : 00 P. M. For soy bean plants grown at a soil moisture of 15 per cent. of the dry weight, the oldest leaves had the highest temperatures, −0.42° C. below the air temperature. Leaves 18 and 10 days old were the coolest, −0.72° and −0.97° C. respectively, below the air temperature. Leaves 4 days old were warmer than those 10 days old, and had a temperature −0.78° C. below that of the air. Whether the increased transpiration rate, as determined by the leaf-temperature determinations, of the leaves 10 and 18 days of age is the result of both stomatal and cuticular transpiration can be determined only by more extensive studies. Likewise it has been reported by a number of investigators that there is an increased respiration rate in the young developing organs, which fact might therefore aid in the explanation of why leaves 4 days of age were warmer than those 10 days old.

The oldest leaves of soy bean plants grown in a soil which had a moisture content of 9.5 per cent. of the dry weight had the highest temperatures. However, instead of being below the air temperature, in all age groups their temperature was above that of the air.

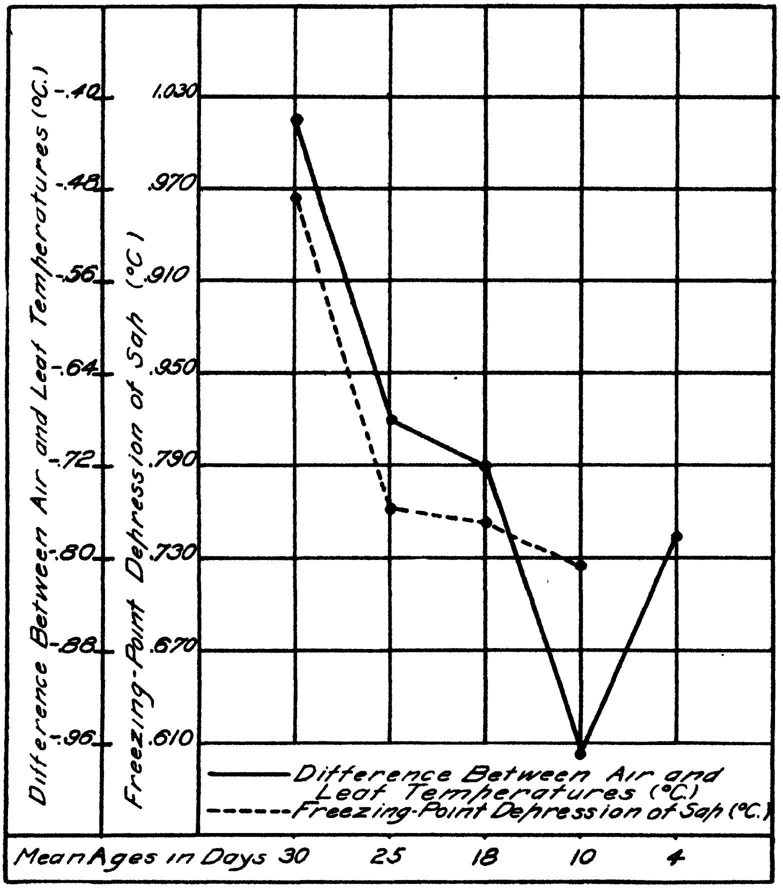

FIG. 2. Ages, temperature measurements, and freezing-point depressions of extracted sap of leaves at successively higher nodes on stem of soy bean plants (variety Manchu, table VI).

The leaf-temperature determinations for the sunflower plants grown at a soil moisture content of 15 per cent. of the dry weight was in the same general order as those of the soy bean plants grown at this soil moisture.

The freezing-point depression increased with the age of the leaves and decreased with the height of their insertion for the plant portions listed in tables VI, VII, and VIII. However, it is probable that this would have been just the reverse if collections of the leaves had been made at 10:30 A. M. rather than at 4:00 P. M. This conclusion is indicated from data presented in table V.

Considering only the osmotic concentration, the upper leaves would be placed at a disadvantage with respect to older leaves in their ability to with-

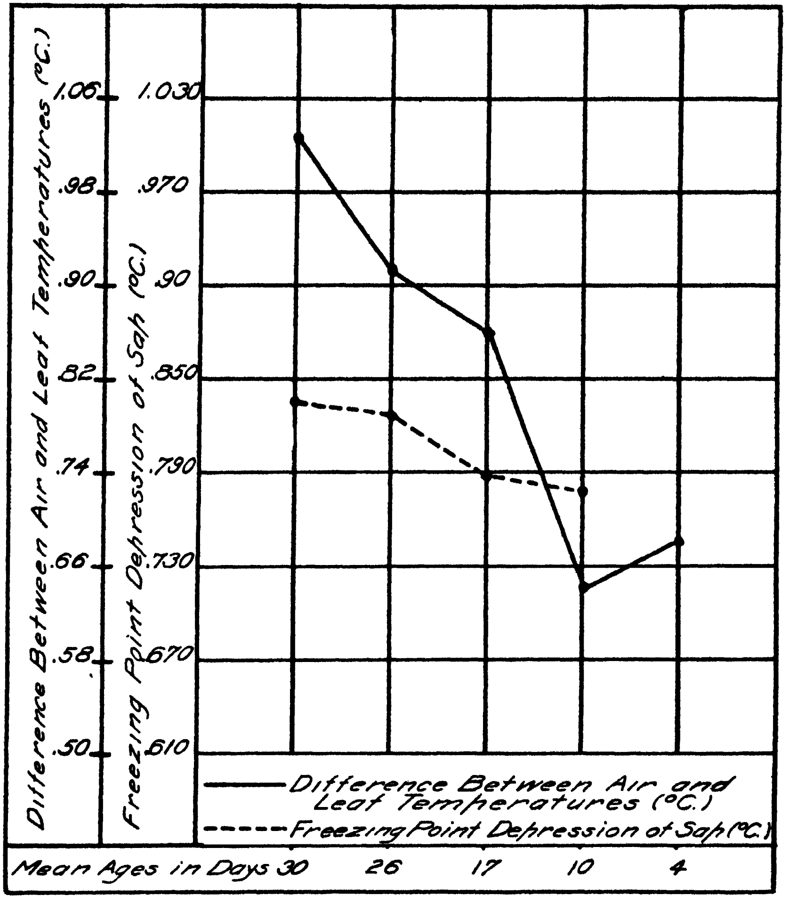

FIG. 3. Ages, temperature measurements, and freezing-point depressions of extracted sap of leaves at successively higher nodes on stem of soy bean plants (variety Manchu, table VII).

draw water from the transpiration stream, at 4:00 P. M. In addition to the difference in osmotic concentration, the upper leaves of soy bean and sunflower were also at a disadvantage in their water relations as a result of their difference in position on the main stem.

It was thought desirable to make a study of the water content of different tiers of leaves of soy bean and sunflower, before wilting and during different degrees of wilting. Table IX presents these data. The plants were grown in pots, and water was withheld to induce wilting. The common method of weighing, drying, and reweighing was employed to determine the water content of leaves. Immediately after picking, the leaves were placed

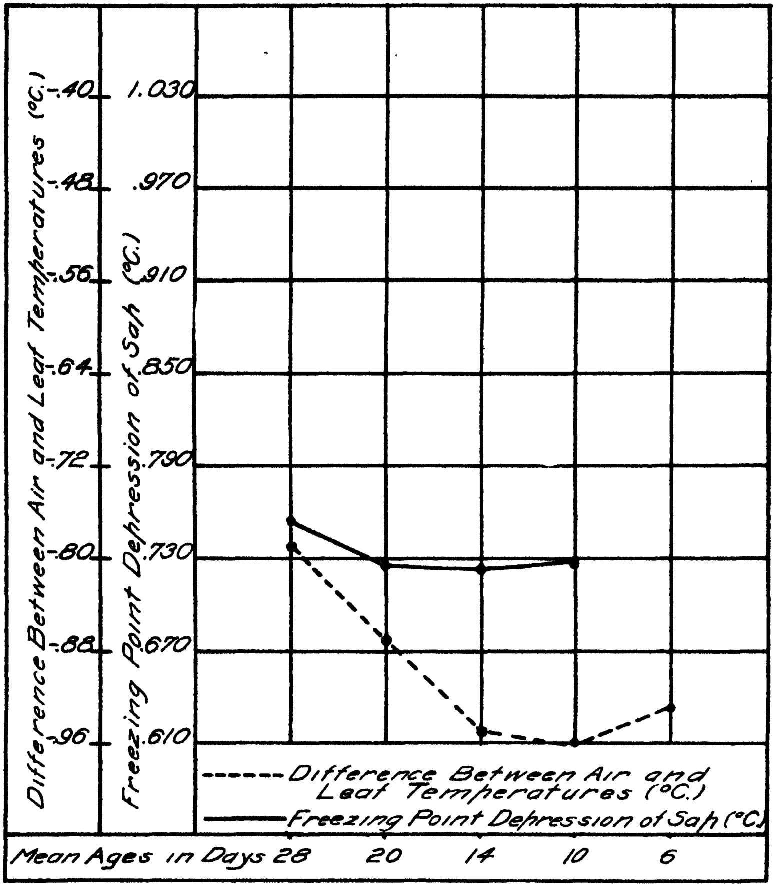

FIG. 4. Ages, temperature measurements, and freezing-point depression of extracted sap of leaves at successively higher nodes on stem of sunflower plants.

in blackened glass tubes which were tightly stoppered. After being weighed the tubes were opened and placed in a drying oven at a temperature of 90°–95° C. The final dry weight was obtained by reweighing the stoppered tubes until they had reached a constant weight.

The results given in table IX show that lower leaves of both soy bean and sunflower have a higher water content than have the upper leaves. Nevertheless the lower leaves either give up some of this water to the upper ones during wilting or it is lost through transpiration, whereas the upper (younger) leaves have some means of retaining their water content.

It may be noted in the case of soy bean (table IX) that the lowest tier of leaves lost 11.75 per cent. of their water content whereas the upper tier lost

TABLE IX

PERCENTAGE OF WATER IN DIFFERENT TIERS OF LEAVES OF SOY BEAN VAR. ILLINI AND SUNFLOWER BEFORE AND AFTER 24 HOURS OF WILTING; TIME OF COLLECTION 3:30 P. M.

DATE	PLANT	TIER OF LEAVES	MOISTURE		WATER LOST
			BEFORE WILTING WAS NOTED	24 HOURS AFTER WILTING WAS NOTED	
			per cent.	*per cent.*	*per cent.*
Nov. 20, 23, 1930	Soy bean	First (lowest)	85.9	75.8	11.76
		Second	85.3	75.1	11.95
		Third	85.3	75.5	11.49
		Fourth	85.2	76.4	10.32
		Fifth	84.1	77.3	8.09
Jan. 21, 23, 1931	Sunflower	First (lowest)	90.3	72.8*	19.38
		Second	90.05	78.6	12.72
		Third	89.5	85.6	4.36
		Fourth	89.08	87.7	2.78
		Fifth	87.5	85.2	2.63
		Sixth	85.07	84.0	1.27

* These leaves had turned slightly yellow near the margin.

only 8.09 per cent. The sunflower plants showed an even greater difference. The lowest tier of leaves lost 19.38 per cent. of their water content, in contrast to 1.27 per cent. for the sixth tier of leaves.

The fact that young leaves retain their turgidity for a longer period than older ones has been explained by PRINGSHEIM (**50**) and others to be the result of a greater osmotic pressure. According to PRINGSHEIM, not only the earlier wilting but also the complete drying of adult parts of plants may be explained by the comparatively low osmotic pressure in these parts.

It is hardly possible to explain the more rapid giving up of water by the lower leaves exclusively by reduced osmotic pressure; for if the upper leaves had a greater osmotic concentration than the lower, as shown when collections were made at 10:30 A. M., the loss of their water content would bring about a greater concentration within the cells, which would therefore arrest the further loss of water. Moreover, the loss of water would be possible only in the case of simultaneous death of young as well as old organs, which apparently does not occur. However, with due recognition of factors which may lead to shifting of equilibria and which must be constantly operative in physiologically active plant cells, we must assume that the movement of water is due only partly to differences in osmotic pressure of the cells; other important factors operating in this connection are probably imbibition pressure and tensile strength of water.

In any transpiring plant there must be a pressure gradient throughout the cell contents to the outside of the cell wall, as well as from cell to cell. As the water vapor diffuses from the intercellular spaces of the leaf to the external atmosphere, the vapor pressure in these spaces is lowered and the liquid water leaves the film on the neighboring cell walls to aid in the establishment of a new equilibrium between the vapor and liquid phases. Therefore, if the rate of evaporation exceeds that of absorption, as in the case of wilting, water which leaves the cell walls cannot be entirely replaced, and thus the concentration of the cell fluids will be increased. In order to maintain this equilibrium, the water film will recede into the pores of the cell walls, the surface force increasing with curvature of the menisci, until an equilibrium is established between the osmotic and imbibition pressures of the cell fluids. Since the logarithmic nature of an imbibition curve means that in a cell containing a high concentration of colloids the pressure goes up very rapidly, therefore the rate of outward diffusion must be slower than in a cell lacking such protection. Since the upper, therefore younger, leaves contain a high percentage of hydrophilic colloids, it is suggested that this condition will aid greatly in the explanation of why the younger leaves in the experiment of table IX were able to retain a greater percentage of their water content during wilting. NEWTON and MARTIN (**45**) point out a close correlation between hydrophilic colloids and drought resistance.

Again in this connection, HARRIS and GORTNER (**20**) found that *Atriplex nuttallii* has a freezing-point depression of 14.4° C., whereas in cactus it is 0.53° C. It was observed by these investigators that *A. nuttallii* did not retain its water as effectively as did the cactus plant. Since their investigation, NEWTON and MARTIN have shown the cactus to have a high hydrophilic colloid content, as determined by the bound-water method.

The unequal distribution of water in the leaves of plants as shown by this investigation substantiates the findings of KRASNOSSELSKY-MAXIMOV (**36**). She likewise noted the water content to be greater in the lower leaves. Her data show that during the daytime, the excess of transpiration over absorption causes the more vigorous parts of the plant, namely, the upper leaves, to abstract water from the lower, less active leaves. On account of this the upper leaves, although subjected directly to the drying influence of sun and wind, were found to exhibit smaller variations in water content than the lower leaves, which are more or less shaded and nearer to the roots.

EFFECT OF PERIODICAL WILTING ON OSMOTIC PRESSURE

The soy beans (data in table X) were grown at about the optimum soil moisture content for a period; then they were periodically subjected to near the point of permanent wilting, after which the plants were once more watered to bring them back to optimum soil moisture. The plants were subjected to severe wilting six times during development, and collections were made when they were 47 days old.

It may be of interest to state that the interval between wilting and death was observed for soy beans, milo, and cabbage. The death of soy beans occurs a few days after wilting, and after many days, even two or more weeks, for milo and cabbage. The number of days before death occurs depends upon a variety of physical conditions. The milo and cabbage are hardier to drought and cold than the soy bean. MAXIMOV (**40**) noted, in his experiments on wilting of plants in relation to drought resistance, that the more drought-resistant plants were capable of losing 30–40 per cent. of their water content without injury, while the less resistant plants showed a reduction in dry weight and shedding of their leaves.

TABLE X

SUMMARY OF DETERMINATIONS MADE FROM COLLECTIONS AT 4:00 P. M. ON SOY BEAN, SUBJECTED TO PERIODICAL WILTINGS; DECEMBER 2, 1930

MOISTURE CONTENT	VOL. OF SAP PER 100 GM. LEAVES	MEAN AGE OF LEAVES	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WAX EXTRACTED
per cent.	*cc.*	*days*	Δ	*atm.*	*per cent.*
75.4		45		.	. .
75.1	39.3	38	1.176	14.152	52.2
75.3	33.3	31	1.033	12.436	46.0
76.0	38.4	25	1.046	12.596	50.1
78.7	43.1	18	1.001	12.052	54.7

The plants subjected to periodical wiltings (table X) had a higher osmotic concentration than the check plants (table XI) grown at approximately optimum moisture content. Further, it may be noted that the water content of the leaves was less in the case of the periodically wilted plants. This difference in water content might account for part of the increase in the osmotic concentration of these plants; however, it is not thought to account for the entire difference. In the check plants the lower leaves contained the greater percentage of water, whereas in the periodically wilted plants the upper leaves contained the greater percentage. Periodical wilting tends to induce a more xeromorphic structure, a higher osmotic concentration, and a possibly greater hydrophilic colloid content within the cells of the leaves, which may in turn aid in neutralizing the unfavorable consequences of the initial wilting, in spite of subsequent exposures to deficiency of soil moisture. Assuming this, wilting during the early stages of development may be actually beneficial, especially during some seasons.

TABLE XI

SUMMARY OF DETERMINATIONS MADE FROM COLLECTIONS AT 4:00 P. M. ON SOY BEAN, 47 DAYS OF AGE, GROWN AT NEARLY OPTIMUM SOIL MOISTURE CONTENT; DECEMBER 2, 1930

MOISTURE CONTENT	VOL. OF SAP PER 100 GM. LEAVES	MEAN AGE OF LEAVES	FREEZING-PT. DEPRESSION OF SAP	OSMOTIC PRESSURE	TOTAL WATER EXTRACTED
per cent.	*cc.*	*days*	Δ	*atm.*	*per cent.*
83.5	51.1	45	0.776	9.346	.
83.3	52.0	41	0.778	9.370	62.5
81.4	52.2	36	0.776	9.346	64.1
80.7	54.5	29	0.739	8.901	67.5
81.1	60.8	23	0.671	8.083	74.9
80.3	63.3	15	0.658	7.926	78.8

EFFECT OF ADDING NACL TO SOIL SOLUTION ON OSMOTIC CONCENTRATION OF PLANT SAPS

A number of investigators have shown that sodium chloride added to the soil solution brings about an increase in the osmotic concentration. HARRIS and PASCOE (**26**) state that there is in general a positive correlation in cotton between the salinity of the soil and the osmotic concentration as measured in terms of freezing-point depression. In order to find whether soy bean of Manchu variety behaved in like manner, the following experiment was carried out. Plants were watered with a solution of 0.05 molar

TABLE XII

EFFECT OF ADDING 0.05 MOLAR SOLUTION OF NACL TO SOIL SOLUTION IN WHICH SOY BEAN PLANTS WERE GROWING; JANUARY 30, 1931

MOISTURE CONTENT	MEAN AGE OF LEAVES	FREEZING-POINT DEPRESSION OF SAP	OSMOTIC PRESSURE
per cent.	*days*	° *C.*	*atm.*
84.2*	38 and 35	1.030	12.400
84.3	29 and 22	1.047	12.604
81.1	20 and 10	1.095	13.180
Check plants			
85.1	39 and 36	0.937	11.284
85.0	31 and 25	0.954	11.488
80.3	22 and 14	0.973	11.716

concentration of NaCl, instead of tap water as for the check plants, in order to keep them at the desired soil moisture. The plants were collected at 9:40 A. M. for freezing and storage.

The results (table XII) show that a 0.05 molar solution of NaCl added to the soil solution increases the osmotic concentration in the case of the soy bean, variety Manchu. The percentage increase amounts to 9.68 when all tiers of leaves are considered together.

EFFECT OF VARYING SOIL MOISTURE ON OSMOTIC PRESSURE OF THE PLANT

Soil moisture has repeatedly been observed to have a marked influence on plant growth. When a plant is grown at or near a minimum soil moisture content, or when subjected to periodical wiltings, its anatomical structure and development are profoundly influenced. Leaves of the soy bean when grown under such conditions were about one-third to one-half as large as those grown under the optimum conditions of soil moisture. The leaves were a darker green, had a denser network of veins, and had a greater number of stomata per unit area. According to MAXIMOV (**40**), these structural changes, which may be termed "xeromorphic," tend to increase transpiration and simultaneously to increase the gaseous exchange. He states, "by virtue of this, xeromorphic plants are distinguished not by a lower but by a higher rate of such processes as transpiration and assimilation."

The percentage of soil moisture is given (table XIII) in tenths (for example, as 9.5 instead of 9.0), because soil samples were taken from time to

TABLE XIII

EFFECT OF SOIL MOISTURE ON FREEZING-POINT DEPRESSION, THUS OSMOTIC PRESSURE; SOY BEAN PLANTS 38 DAYS OLD; COLLECTED AT 4:00 P. M.

DATE	VARIETY	CONCENTRATION BY REFRACTION	FREEZING PT.-DEPRESSION OF SAP	OSMOTIC PRESSURE	SOIL MOISTURE
1930		*per cent.*	Δ	*atm.*	*per cent.*
Nov. 4	Manchu	7.4	0.813	9.791	9.5
Nov. 4	Manchu	7.1	0.809	9.743	10.4
Nov. 4	Manchu	6.5	0.801	9.647	11.8
Nov. 5	Illini	5.7	0.711	8.654	13.7
Nov. 4	Manchu	6.8	0.789	9.502	15.0
1931 Jan. 21	Ebony	5.3	0.746	8.985	15.0
Jan. 21	Ebony	4.5	0.728	8.768	16.2
Jan. 21	Ebony	4.6	0.715	8.612	17.0

time and the percentage of water determined. Therefore when all of the results of the samples were averaged together, it gave 9.5 rather than 9.0 per cent. None of the soil samples taken varied more than ± .9 of 1 per cent. These data show that the osmotic pressure varies with physiological scarcity of water. It is probable that still more marked differences might be obtained by the use of other plants.

The soy bean has not been found to be very drought-hardy. Likewise, these plants were grown under a constant soil moisture and a nearly uniform temperature and humidity, which in all probability would influence the osmotic concentration. As previously pointed out, the subjection of a plant to periodical wiltings increases its osmotic concentration and probably its hardiness against further loss of water. Greater difference in osmotic concentration has been pointed out between the preceding range of soil moistures; however, they were determinations made on plants grown out of doors, where they were subjected to great changes in soil moisture, humidity, and temperature. These changes tend to increase the osmotic concentration.

DRABBLE and DRABBLE (**11**) were apparently the first to investigate the relation between osmotic pressure of the cell sap in plants and soil moisture. They found it to vary directly with the scarcity of water. Thus the present results substantiate their observations as well as those of some later investigators, previously mentioned.

GEBHARDT (**18**) has recently stated that the saps of plants of different systematic and ecological groups grown under the same soil conditions have about the same osmotic pressures. The results of NEWTON and MARTIN (**45**) do not substantiate this statement, and there are no marked indications of this in the present investigation.

VARIETAL DIFFERENCES IN SOY BEANS

In soy beans there is a complete series of varieties ranging from very early to very late maturing ones. Observations have been made as to the comparative behavior of Ebony, Manchu, and Mandarin varieties with respect to hardiness when grown under partially controlled conditions.

The temperature of 75°–76° F. appears to be approximately optimum. All varieties were found to be sensitive to cool temperatures. A few pots of each variety, of the same age and grown under identical conditions, were placed in a lighted room at 38° ± 2° F. for a period of 72 hours, to see whether any difference could be noted in the varieties. They showed about an equal degree of sensitiveness to this temperature; however, Manchu and Mandarin showed a smaller degree of injury than Ebony. SELLSCHOP and SALMON (**54**) found that Virginia soy beans were severely injured by an exposure of 72 hours, whereas Manchu survived an exposure of 120 hours without injury, when subjected to a temperature of 0.5°–5° C.

It was likewise desirable to know whether these varieties differed as to drought hardiness. A number of pots of each variety were planted and kept at constant soil moisture until they had attained a height of about 8 inches; then they were placed upon a table and allowed to wilt severely before adding enough water to bring back the required soil moisture. The plants were permitted to wilt severely at three different times. After the last wilting they were left unwatered for 7 days. At the end of this period the soil in the pots was dry and the plants in a state of permanent wilting, several having perished. Water was again added to the soil, to permit the plants not already dead to recover. After a few days, record was made of those that had died and of those that had survived. It should be stated that severe wilting greatly reduces the yield, the percentage of which, however, was not determined in these plants. The results are given in table XIV.

TABLE XIV

ENDURANCE TO WILTING OF DIFFERENT VARIETIES OF SOY BEAN PLANTS

VARIETY	NUMBER OF PLANTS		SURVIVAL PERCENTAGE
	IN EXPERIMENT	WHICH SURVIVED	
Manchu	24	10	41.7
Ebony	18	6	33.3
Mandarin	21	11	52.4
Harbinsoy	19	8	42.1
Illini	11	5	45.4

The results (table XIV) show no marked difference between the varieties; however, Mandarin showed a slightly higher percentage of survival than any of the others. Since this is an early variety, possibly it has the potentiality of building up substances which aid it better to withstand drought conditions, although this can be determined only by more extensive studies.

In a variety trial on frost resistance at Muskegon, Michigan, in 1909 (**48**), the comparative resistance of varieties was reported as follows, the first being least injured: (1) Ogemaw; (2) Haberlandt, Ito San, Kingston, Guelph; (3) Habaro, Shingto, Manhattan, Brindle; (4) Jet; (5) Meyer, Chernie. Since the Ogemaw is a very early variety and was found to be the most resistant to frost, there may be a correlation between early varieties and resistance to cold and drought. Maturity of the plant is probably an important factor in either case, and one might expect the same variety to vary in cold and drought resistance, depending upon its stage of maturity.

Only slight variations of the osmotic concentration have been observed for the different varieties when grown under as nearly identical conditions as possible. This is probably to be expected since osmotic values have been found to be largely the result of the environment. However, it is not intended to discount the importance of osmotic properties in the water relations of plants. This point will be touched upon again in connection with the bound-water studies.

V. Bound-water studies in cabbage and milo

There are undoubtedly many factors concerned in the ability of plants to survive a drought or a cold period; but, above all, the potentiality of a plant to build up hydrophilic colloids seems to be of the utmost importance. NEWTON and MARTIN (**45**) have recently pointed out the importance of these substances in relation to drought resistance. ROSA (**52**), in his studies on vegetable crops, found that the hardening process was accompanied by a marked increase in the water-retaining power of the tissues, due primarily to an increase in the imbibitional forces of the cell. He found that pentosans accumulate in cabbage and a few other vegetables during the "hardening off" process, and that this accumulation may be brought about either by exposure of the roots to deficient soil water for typical growth or by exposure to low temperatures. He used the dilatometer freezing method of FOOTE and SAXON (**16**) to determine the quantity of "free" and "unfree" water in the tissue. This method is an indirect measurement of the sum of the osmotic and imbibition pressure of the plant cells.

NEWTON and BROWN (**44**) found that the percentage of proteins and pentosans in the total dry substance of wheat plants does not increase in the fall during the hardening process, and that the marked rise in the concentration of these substances in the extracted plant juice is due to a reduction in the moisture content of the tissue. These investigators found the proteins to constitute the bulk of cell colloids, pentosans being restricted almost entirely to the structural parts of the plant. It is altogether probable that pentosans of the cell wall play a rôle in drought and cold resistance. As has been previously pointed out in connection with the ability of the younger leaves to retain their water content during wilting, these water binding substances may be in different quantities in old and young cell walls. DOYLE and CLINCH (**10**) also found that there is no apparent connection between cold resistance and pentosan content of the leaves of conifers. Thus the work of these investigators indicates that pentosans play little if any part in cold and drought resistance of certain plants.

Further experiments have been carried on to determine the effect of soil moisture on the percentage of bound water in milo and cabbage. The bound-water method for the cabbage plant has been checked against the "hardening off" process by low temperatures. The low temperature method has been used by HARVEY (**28**) and others. Cabbage plants were used because they can be hardened easily, the evidences of freezing injury can also be readily seen, and varieties with known and relatively uniform hardening capacity are available. Milo is also known to be resistant to drought. The milo seed was secured from the University of Arizona. Four varieties of cabbage were used: Early Jersey Wakefield, Charleston Wakefield, Copenhagen Market, and Late Drumhead. Only a few varieties are adapted for early spring and fall planting. In the region of Norfolk, Virginia, the Jersey Wakefield, Copenhagen Market, and Charleston Wakefield have been found superior in winter resistance; therefore studies have been made to determine the reasons for this superiority in winter resistance and to test the possibly important factors of drought and cold resistance.

Two constant temperature chambers were used for securing the low temperatures desired. These chambers were equipped with accurate electrical temperature controls and the low temperatures established by refrigeration. A light was left burning in the chambers so that etiolation did not occur during the "hardening off" process. Great care was taken to eliminate differences in exposure of the plants to this light.

Instead of pre-freezing the tissue used (table XV) with solid carbon dioxide, the method of grinding in a food grinder was used before extraction under pressure. The results presented show in general that cabbage and milo when grown in a soil with abundance of available moisture exhibit a smaller tendency toward the development of hydrophilic colloids, thus the decreased percentage of bound water; whereas when the plants are provided with less available soil moisture or subjected to periodical wiltings the onset of drought conditions causes a greater proportion of the water in the cells to be changed from a free to a bound state. NEWTON's (**20**) data indicate that the elaboration of the hydrophilic colloids does not take place under greenhouse conditions, but is the result of subjecting the plants to a period of progressively decreasing temperatures. ROSA's (**52**) work shows that pentosans accumulate in cabbage as a result of depriving the roots of available moisture. The present findings substantiate those of ROSA, since the hydrophilic colloids are increased, as indicated by the increase in percentage of bound water when the plants were subjected to periodical wiltings.

The data given under "concentration" in table XV indicate considerable increase of dry substance in plants exposed to drought "hardening off" conditions. This is especially noticeable in the more hardy varieties, as Early Jersey Wakefield. Milo also shows a considerable increase in dry substance. In general the water content may be said to decrease in drought

TABLE XV

DATA ON CABBAGE AND MILO GROWN IN THE GREENHOUSE AT DIFFERENT SOIL MOISTURE CONTENTS

DATE (1931)	PLANT	AGE OF PLANT	SOIL MOISTURE	CONCENTRATION BY REFRACTION	FREEZING-PT. DEPRESSION	BOUND WATER
	Cabbage	*days*	*per cent.*	*per cent.*	Δ	*per cent.*
Mar. 3	Charleston Wakefield	45	15.9	8.7	1.087	5.22
Mar. 3	Copenhagen Market	45	15.9	8.1	0.964	5.35
Mar. 5	Late Drumhead	47	15.0*	8.6	1.022	5.13
Mar. 5	Early Jersey Wakefield	47	15.0*	8.2	0.892	6.53
Mar. 10	Early Jersey Wakefield	52	14.0	8.4	1.072	7.90
Mar. 10	Late Drumhead	52	14.0	8.9	1.139	6.80
Mar. 13	Late Drumhead	55	Periodically wilted	10.6	1.335	6.10
Mar. 13	Charleston Wakefield	55	" "	10.2	1.347	7.30
Mar. 13	Early Jersey Wakefield	55	" "	11.0	1.208	9.53
Mar. 17	Milo	40	" "	8.9	1.069	6.89
Mar. 17	Milo	40	15.0	7.8	0.690	4.30
Feb. 9	Milo	62	17.0	6.2	0.659	3.61

Coefficients of correlation	*n*	*r*
Concentration and osmotic pressure	12	0.94 ± 0.022
Concentration and bound water	12	0.66 ± 0.109
Osmotic pressure and bound water	12	0.54 ± 0.13

* An equal number of plants chosen from chambers of 14, 15, and 16 per cent. soil moisture.

and cold-hardened plants in proportion to the extent to which their growth is checked by the hardening treatment. It is obvious that any hardening process greatly influences growth. This has been noted previously in the study of periodical wilting.

The depression of the freezing point was somewhat greater for the drought-hardened plants. Similar results have been obtained by other investigators, as CHANDLER (7), HARVEY (27), ROSA (52), and others, most of whom worked with cold-hardened plants. The differences found here are somewhat greater than those found by HARVEY and CHANDLER. This is perhaps due to the severe treatment used.

One of the most important factors involved in the increased osmotic concentration of hardened plants is the decrease in moisture content; another is the increase of sap solutes, as sugars; and, lastly, an increased amount of water in the bound state. A number of investigators have found an increase of soluble sugars in plants exposed to low temperatures, and recently ILJIN (**31**) reported this to be true for plants growing in very dry habitats.

It may be pointed out that both drought- and cold-hardening processes in plants are accompanied by a marked increase in the water-retaining power of the cells that enables them upon freezing to retain a large proportion of their water content in the unfrozen condition or state. Figure 5 shows four plants of two different varieties, Late Drumhead and Copenhagen Market: (1) Copenhagen Market hardened by exposure to severe wiltings; (2) Copenhagen Market hardened by placing in a cold chamber which was maintained at 3.5° C. for 5 days; (3) Late Drumhead, given treatment identical with that of no. 1; (4) Late Drumhead, given same treatment as that of no. 2. These plants after being hardened were placed in a cold chamber, which was maintained at a temperature of −4.5° C. for 18 hours. They were then transferred back to a chamber at 3.5° C. for a few hours, before removing to the greenhouse. The plants were photographed the following day. These facts afford further evidence of a close internal connection between the phenomena of freezing and desiccation.

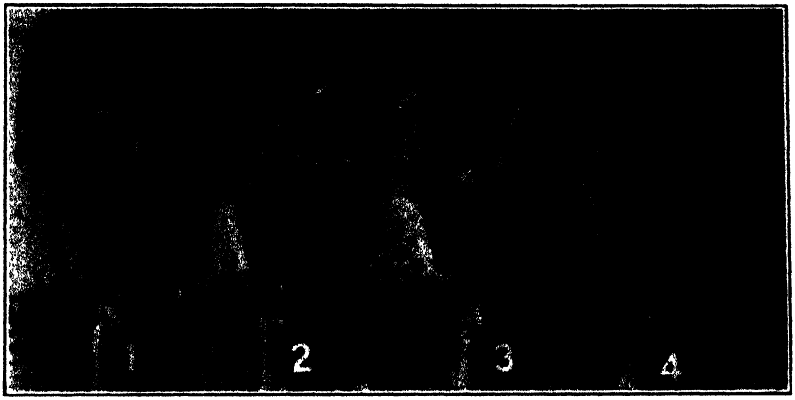

FIG. 5. Showing results of drought and cold hardening: nos. 1 and 2 are Copenhagen Market; nos. 3 and 4 are Late Drumhead. Nos. 1 and 3 hardened by exposure to severe wiltings; nos. 2 and 4 hardened by placing in cold chamber.

Hardy varieties of cabbage possess the potentiality to initiate these changes which aid in retaining the water content of the cells against desiccation, whether it be by freezing or drying. However, there seems to be a slight difference in ability, even among so-called hardy varieties. Cabbage and milo harden readily, and certain varieties of cabbage can withstand drought and quite low temperatures, but soy bean seems to lack this potentiality.

Figure 6 shows the differences in the varieties of cabbages to withstand low temperature. The plants shown in this photograph were representative ones chosen from those studied on March 13 (table XV), with the addition of the variety Copenhagen Market. This comparison of the two methods for the study of hardening in plants was made to see whether any correlation existed between the percentage of bound water, as determined by juice analyses, and the ability of these varieties to withstand low temperature. The plants shown in figure 7 were placed in the chamber maintained at a temperature of −4.5° C. for 12 hours. The photograph was taken the following day. Plants nos. 1 and 2 are Charleston Wakefield; nos. 3 and 4 are Copenhagen Market. Sap analyses were not made on these latter plants, but they were given identical treatment with those compared. Nos. 5 and 6 are Early Jersey Wakefield. There is a correlation between the two methods of determining the ability to retain their water contents. The Early Jersey Wakefield plants were the only ones that survived this treatment and continued their growth processes.

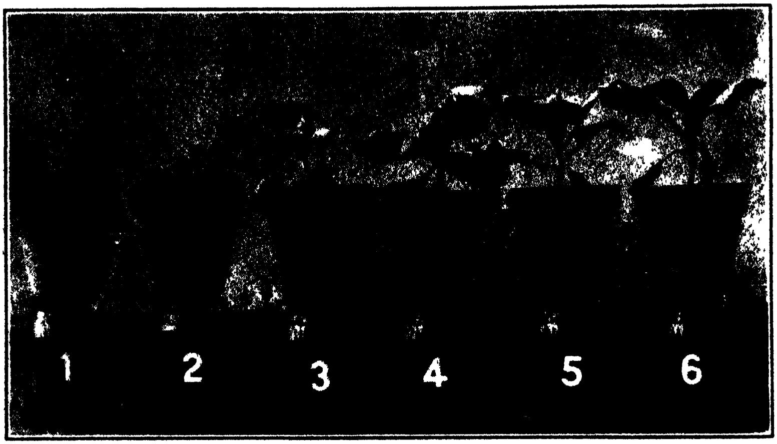

Fig. 6. Showing differences in the varieties of cabbages to withstand low temperatures: nos. 1 and 2 are Charleston Wakefield; nos. 3 and 4 are Copenhagen Market; nos. 5 and 6 are Early Jersey Wakefield.

Figure 7 shows the effect of variation in soil moisture on cold resistance of two varieties of cabbages. Plants nos. 2, 4, and 6 are Early Jersey Wakefield; and nos. 1, 3, and 5 are Late Drumhead. Plants 1 and 2 were grown in a soil with 14 per cent. moisture; nos. 3 and 4 with 15 per cent.; and nos. 5 and 6 with 16 per cent. The plants were subjected to a temperature of 3.5° C. for 5 days, then transferred to a temperature of −4.5° C. for 12 hours. All of the plants died in a few days, with the exception of the

Early Jersey Wakefield plants which were grown at 14 per cent. soil moisture, and these finally died after a period of two weeks. Both methods of study place the varieties of cabbages used in the order named, so far as hardiness is concerned: Early Jersey Wakefield, Copenhagen Market, Charleston Wakefield, and Late Drumhead.

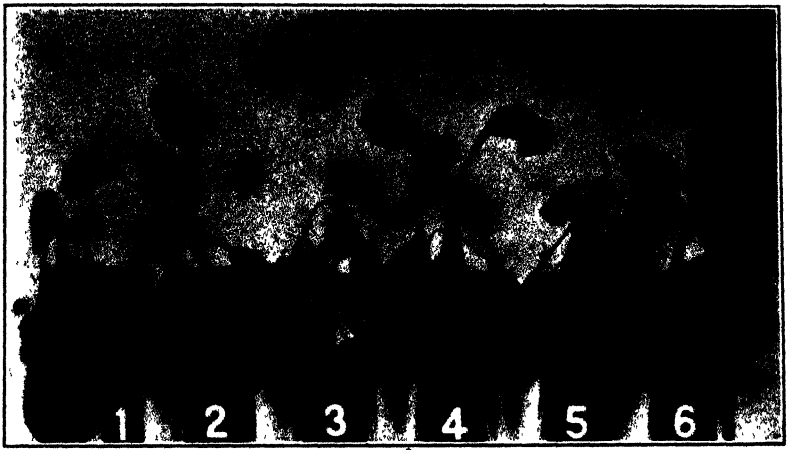

Fig. 7. Effect of variation in soil moisture on cold resistance of two varieties of cabbage: nos. 2, 4, and 6 are Early Jersey Wakefield; nos. 1, 3, and 5 are Late Drumhead.

Recently Harvey (**29**) studied the effect of different treatments for hardening plants, using a single uniform variety of cabbage. From this investigation he suggests that hardiness in plants is a cold shock response.

VI. Correlations of various factors

Freezing-point depression and leaf temperature

Values for the freezing point of the extracted sap and the leaf-temperature departures were obtained for leaves in age groups from 30 to 4 days; these data are presented in tables VI, VII, and VIII. The leaf temperatures and freezing-point values for these groups are found to be correlated to the extent of -0.61 ± 0.15. The correlation is not marked and does not indicate a very close relationship between the changes in leaf temperature and the concentration of the extracted sap. Livingston (**37**) and Drabble and Drabble (**11**) pointed out that an increase in the concentration of a solution does not reduce the vapor pressure enough to reduce effectively the rate of evaporation. On the other hand, Eaton (**12**) found a high correlation between leaf temperature and depression of freezing point, and he concludes that low sap concentrations are conducive to high transpiration rates and reduced leaf temperatures. It should be realized that his correlations are based on leaves studied on one date and time, namely, September 2, between 2:17 and 2:47 P. M. Data of table V show that if collections are made at different times in the day the gradient of the osmotic concentration will be greatly influenced; however, in none of the determinations on leaf temperature were older leaves found to be cooler than the younger (upper) ones.

Any mechanism which limits evaporation makes high osmotic pressure less necessary. Data in table IX show that young (upper) leaves retain their turgidity for a longer period than older (lower) ones. As previously stated, it is believed that osmotic pressure plays only a small part in this phenomenon. None of the data in this investigation seems to substantiate EATON's conclusions on the relationship between extracted sap and leaf temperature and that osmotic pressure plays the important part in retaining the water within the leaf cells.

The coefficient of correlation *r* between the properties measured, and the number of cases *n* on which they are based, are given for the cabbages and milo at the end of table XV. The properties measured were concentration of the juice, osmotic pressure, and percentage of bound water.

The concentration of juice and osmotic pressure are found to be correlated to the extent of 0.94 ± 0.022; the concentration and percentage of bound water to the extent of 0.66 ± 0.109; and the osmotic pressure and bound water to the extent of 0.54 ± 0.13.

There seems to be no marked correlation between osmotic pressure and percentage of bound water. This indicates that while both of these factors may be related to drought and cold hardiness, they cannot both be a direct index to this phenomenon.

It was possible in most cases to classify the varieties of cabbages as to their ability to withstand low temperature by determining the percentage of bound water in the extracted juices. On the other hand, osmotic pressure afforded no marked indication of ability to withstand low temperature. Frequently the osmotic pressure would be greater for the variety which gave the lowest percentage of bound water and for the plants which were the most easily affected by the low temperature. Osmotic pressure is therefore probably not characteristic, but controlled mainly by habitat conditions. The soy beans and cabbages that were grown under nearly identical conditions showed only slight variations in osmotic pressure. It is strongly indicated that osmotic pressures are largely the result of environment.

The percentage of bound water of the fresh juice seems to be the best index of drought and cold hardiness, with concentration of the juice showing considerable relation to moisture adaptations. It is not intended to discount the importance of osmotic pressure in the water relations of plants, but this relationship has probably been over-emphasized. There seems to be little doubt that osmotic pressure and imbibition pressure are closely connected in the water relations of plants. There are also some indications that osmotic pressure varies characteristically in certain ecological types; it seems to be slightly higher in the soy bean and cabbage than in the sunflower when grown under as nearly identical conditions as possible. It should also be pointed out that the percentage of water seems to be influenced by the percentage of soil moisture in which the plants are grown. However, the percentage of bound water seems to be more stable than the osmotic pressure.

VII. Summary

1. The more important methods which have been used in the treatment of leaves before the extraction of tissue fluids are presented. Experimental data are given in an attempt to evaluate these methods as to their advantages and disadvantages.

2. The moisture content may be read approximately by a refractometer, provided there is applied a suitable correction which has previously been determined experimentally.

3. Extracted plant juice stored at 0° and 20° C. increased gradually in osmotic pressure, as determined by the freezing-point depression, with time and the respective temperatures. The technique followed in this investigation largely avoids any errors which might be due to these causes.

4. With the approach of maturity, the moisture content of the leaf tissue decreased rather rapidly, causing corresponding increase in concentration and osmotic pressure of the sap.

5. The osmotic gradient within the plant was found to be just the reverse, when collections were made at 10:30 A. M. and 4:00 P. M. respectively. The freezing-point depression of the extracted tissue fluids decreased from the older leaves to the younger, for the material collected at 4:00 P. M.; whereas it increased from the older leaves to the younger, for the material collected at 10:30 A. M. A partial explanation for this difference is proposed.

6. The leaf-temperature measurements were made by the thermo-electrical method, in terms of the departures of the leaf temperature from the temperature of the air. The younger leaves were found to be cooler than the older ones. The leaf temperatures and the freezing-point values were found to be correlated to the extent of -0.61 ± 0.15. This correlation is not marked and does not indicate a very close relationship between the changes in leaf temperatures and the concentration of extracted sap.

7. Periodical wilting has a marked effect upon plant growth, structure, and properties of plant juice.

8. No marked differences were noted in varieties of soy beans as to osmotic concentration, ability to withstand wilting or chilling, and time of flowering, when grown under nearly uniform conditions.

9. Determinations were made of the concentration of the juice, osmotic pressure, and bound water of expressed sap of the leaves of milo and different varieties of cabbages. The percentage of bound water was determined on the juice of these cabbages, and at the same time a few representative plants were placed in a cold room. The two different methods of treatment show a high correlation.

10. On the basis of bound-water studies, elaboration of the hydrophilic colloids is thought to occur under greenhouse conditions, when the plants are subjected to periods of progressively decreasing soil moisture.

11. The varieties of cabbages could be classified very satisfactorily with respect to withstanding low temperature, on the basis of the bound-water content of the fresh juice.

12. It is believed that a high colloidal content reduces the rate of abstraction of water from the leaf tissue when exposed to low temperature or high transpiration rates, and probably confers upon these leaves the ability to withstand desiccation caused either by evaporation or ice formation.

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UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS.

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COMPOSITION OF CURRENT AND PREVIOUS SEASON'S BRANCH GROWTH IN RELATION TO VEGETATIVE AND REPRODUCTIVE RESPONSES IN *PYRUS MALUS* L.[1]

WALTER THOMAS

(WITH FIVE FIGURES)

I. Introduction

The purpose of this experimental project was to determine the response of apple trees to different combinations of the principal fertilizer constituents, nitrogen, phosphorus, and potassium, but with the elimination of the variables usually present in most field experiments. The phase of the investigation reported in this paper is concerned with the association of vegetative and reproductive responses to the internal conditions as represented by analysis of metabolically active tissues at different periods in the growth cycle.

1. PRINCIPAL NUTRIENT ELEMENTS

Although, as is pointed out by LIVINGSTON (**96**), it is conceivable from the physiological viewpoint that conditions may be found under which any element other than the traditional ten is essential to growth, experience has shown that cultivated plants are, under ordinary field conditions when the percentage base saturation (**152**) is not the limiting factor, far more sensitive to changes in the nitrogen, phosphorus, and potassium content of soils than to any of the other elements. There is evidence (**2, 139, 165**) that the peculiar and characteristic effects of nitrogen, phosphorus, and potassium may be due to the greater motility of their ions and, in the case of potassium, to the effect on the streaming rate of protoplasm. COLLA (**30**), for example, finds that potassium has a greater effect in increasing the streaming rate of the protoplasm of *Chara crinita* than sodium or magnesium. But, whatever may be the mechanism of their action, the existence of the fertilizer industry is sufficient evidence of the agricultural economic importance of these three elements. From the standpoint of behavior and function, there is great need for more definite information concerning the rôle played by each of these dominant nutrient elements with respect to growth, reproduction, and other physiological functions.

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2. SEQUENCE OF DISTRIBUTION OF VARIATIONS IN CONCENTRATION OF ELEMENTS WITH RESPECT TO TIME

The experiences of the last decade (**18, 46, 47, 69, 85, 86, 87, 88, 89, 90, 91, 174, 201**) have confirmed and extended the value of the earlier investigations (**230, 231**) in which the concentration of certain elements or groups of elements was determined in the metabolically active tissues at critical periods in their vegetative development. Investigations of this type have proved of special service in the diagnosis of the nutritive conditions produced in plants by the application of *different combinations* of the principal nutrient elements. In this way we may describe metabolic processes with reference to their sequence of changes with respect to time. Although this method of approach does not necessarily enable the "causal relations" (**97**) to be determined but probably only the "residual effects" (antecedent influential conditions), the knowledge obtained from studies of this type supplies a valuable picture of the dynamics of physiological processes. Experiments planned in this manner have already enabled the investigator to detect quantitatively the lack of optimum (well-balanced) nutritive conditions and to prescribe remedial measures (**208**).

The sequence of changes with respect to the chemically reactive ions or reactive groups determined in this manner is not sensibly influenced by the range of variation in the meteorological conditions hitherto encountered in experiments with fruit trees. This is indicated by the similarity in direction of and relative magnitude of the seasonal changes of reactive groups or complexes, such as glucose, sucrose, starch, and also of the elements potassium and phosphorus in the present experiments compared with those reported under widely different meteorological conditions for the same species by others (**69, 122**). The writer has also obtained indications (**201, 202**) that the seasonal variations (*i.e.*, the time-concentration-curve) of the various nitrogen fractions throughout a vegetative cycle have also been affected primarily by internal causes.

3. MECHANISM OF FERTILIZER ACTION

The present investigation is characterized by a remarkably rapid but differential response of the plants to relatively small applications—low concentrations—(page 396) of the elements nitrogen, phosphorus, and potassium, the response varying according to the combination in which these elements are present. Marked differentiation between the trees of certain treatments was apparent even within a period of six weeks after application of the nutritive elements, and the differences in response became more intensified as the experiment progressed. How do such small quantities have such marked results? How have these trees grown so differently? Is the mechanism of fertilization one of compensation or of stimulation?

According to LIEBIG's (**94**) "mineral theory," fertilizers are effective only in so far as they supply to the soil the mineral elements in which the soil is relatively deficient. In 1903 WHITNEY and CAMERON (**224, 225**) stirred the scientific world by the announcement that plants obtain their nutrients from the substances *already existing in the soil solution.* The theory was advanced that the soil solution was approximately constant in composition, *viz.*, a saturated solution of the minerals common to all soils, and that accordingly the soil solution was in *simple equilibrium* with the soil-forming minerals. The addition of fertilizers, therefore, according to these investigators, could not increase the concentration of the soil solution. Any response to applications of nitrogen, phosphorus, or potassium by plants was attributed by WHITNEY and CAMERON to their action on the physical properties of the soil, to their effect in neutralizing toxins, and also to their catalytic or stimulatory action. This view was vigorously disputed, especially by HOPKINS (**73**), and also by the Rothamsted group (**56, 168**) who showed the fallacies of this conception. Experimental evidence has now accumulated showing that the concentration and composition of the soil solution are continuously changing (**20, 66, 177, 186, 217**), being modified by several factors: (a) the amount of soil moisture, (b) the rate of absorption and utilization of elements by the plant, (c) the respiratory coefficient of the roots, and (d) the activity of the microorganisms of the soil and of the rhizosphere.

The theory of *activation* as opposed to *compensation* has recently been revived by Italian investigators, especially by MAGRINI (**108**). The latter concludes that fertilizers act to stimulate the plant to absorb certain other nutrients found in the soil. But as the sole evidence submitted in support of the theory is the observation that only a negligible quantity of the nitrogen of the subsoil is removed by the crop after each cultivation, this would seem to be insufficient to justify the conclusion.

4. EFFECTS OF SALTS ON THE SOIL SOLUTION

Numerous investigators (**43, 51, 182, 213, 218**) have shown that fertilizer salts produce highly complicated effects. They may cause marked changes in the composition of the soil solution. For example, it has been shown by the writer (**197**) that the addition of $CaH_4(PO_4)_2$ to the Hagerstown silty clay loam soil used in these experiments increased the Fe and Al in the soil solution, and that $NaNO_3$ and K_2SO_4 increased the amount of PO_4 in solution, whereas both $CaH_4(PO_4)_2$ and K_2SO_4 increased the amount of Na in solution. The effect of fertilizers, accordingly, will necessarily depend on the resultant effect on the dominant nutritive ions of the soil solution (**208**). The reciprocal effects of nitrogen, phosphorus, and potassium in relation to their absorption by plants has been treated in detail by the writer in another paper (**208**).

5. DO ROOTS DERIVE THEIR NUTRIENTS FROM A SOLUTION MUCH MORE CONCENTRATED THAN THAT OBTAINED BY DISPLACEMENT METHODS?

This question is of paramount importance, for upon its answer depends the validity of the interpretation of the results of experiments which have for their purpose the determination of the critical concentrations of essential ions, *i.e.*, the concentration with respect to any element or ion below which the total absorption by the plant per unit of time is inadequate.

Many plant physiologists suggest that the chemotropic property possessed by the fine roots causes the root to grow from regions of low concentration of solutes to regions of higher concentrations. These regions of high concentrations would, presumably, be produced initially by the action of the CO_2 and other acids formed in the metabolic processes of the microorganisms growing around the colloidal and mineral complex of the soil particles (**103**). The mechanism involved may be explained in part at least by the GIBBS-DONNAN law (**34, 35, 208**) and in part by the processes discussed in detail by MATTSON (**116, 117, 118, 119, 120**). Diffusion processes then would result in the formation of a series of concentric zones of progressively decreasing concentration of solutes. As the plant grows the carbon dioxide evolved in the respiration of the roots becomes the dominating factor in the dissolution of the soil particles (**207**), furthering the formation of these concentric zones of decreasing concentration. It follows that one of the fundamental problems in studies on the *interrelationship between plant and soil* is the question whether plants can or cannot absorb colloids (**204**), and whether the actual soil solution can be obtained by any of the numerous procedures proposed to achieve this purpose, such as water extraction (**17, 19, 68, 217**), pressure (**95, 125, 162, 216**), displacement (**31, 74, 148, 171, 216**), centrifugal (**15, 16**), and freezing-point (**64**) methods.

An examination of the data by BURD (**17, 19**), HOAGLAND (**64, 67, 68**), MILLAR (**121**), PARKER (**148, 149, 153**), STEWART (**186**), TEAKLE (**195**), and also by the writer (**197**) indicates that the soil solution obtained by displacement methods gives in the case of slightly acid or neutral soils a rather close index of the magnitude of the critical concentrations for different plants of the principal nutrient ions and of the supplying power of the soil with respect to these ions. It has frequently been observed (**19, 52, 149**), however, that crop growth is not related to the concentration of phosphate in the soil solution. For a critical analysis of the anomalous position of phosphorus the reader is referred to papers by BURD and MARTIN (**21**), GREENHILL (**52**), and TIDMORE (**209**).

It is of interest to note that the critical concentration for PO_4 may be as low as 0.20 p.p.m. in flowing cultures for corn (**149, 209**), and the critical concentration for K in the case of alfalfa and clover may be under 0.5 p.p.m., although relatively higher values for some plants are reported, *e.g.*, 1 p.p.m. PO_4 for barley (**67**) and over 4 p.p.m. K for tomatoes (**78**). It must be noted, however, that TRELEASE and LIVINGSTON (**212**) have cautioned against the attempt to determine the comparative physiological values of different nutrient solutions without a knowledge of the climatic conditions under which the tests are made.

Specific soils, of course, vary in their ability to renew a particular nutrient in the soil solution at suitable levels of concentration, *i.e.*, above the critical concentration; and this power of renewal will depend upon the resistance to decomposition of the mineral particles and to the nature of the colloid complexes. It is necessary, too, to distinguish between the two forms of soil solution: the micellar solution, being an integral part of the micelle itself, cannot be renewed.

6. ABSORPTION OF MINERAL ELEMENTS

In earlier papers the writer (**204, 205, 206, 207, 208**) has discussed some of the factors determining the absorption of nutrient elements by the plant. The effect or action of a specific ion under given conditions of light and temperature as related to its absorption and utilization within the plant is dependent upon the concentration of the media, upon the nature of the other ions present, and upon the ratio of the concentrations of equally charged ions to one another.

There are a number of experiments (**65, 109, 163, 196**) which indicate that variations in the *total concentrations* of ions may have less effect on metabolism than changes in the *proportion* of the ions. Since continual fluctuations in the concentration and ratio of the nutritive ions in the soil solution occur, it might be expected that by the processes of adaptation plants would be gradually adjusted to somewhat wide variations in this respect; or, as HOAGLAND (**67**) expresses it, "there is no doubt that all kinds of interionic effects occur during absorption, varying with the composition and concentration of the culture solution, stage of growth and climate."

The soil conditions involved in an adequate understanding of the problems connected with the interrelationship of the plant and soil have been briefly sketched here in order that the reader may keep in mind the significance of the substrate in relation to the facts brought out in this paper. Only the analyses of the plant are reported; the soil conditions have been discussed in previous papers (**198, 208**).

II. Materials and methods

1. SOIL USED

During the period 1918–20, the late WILLIAM FREAR and the writer made several attempts to obtain information regarding the effect of the principal fertilizer elements on the internal conditions of apple trees growing in the experimental orchards then conducted by the Pennsylvania State College throughout the State. But, inasmuch as no consistent results could be obtained, this plan was abandoned for one in which soil and also stock variations were eliminated. This was accomplished by the use of boiler-plate cylinders, 42 in number, 5 feet in diameter and 5.5 feet deep, which were sunk into the ground and filled with a virgin soil (a silty clay loam of the Hagerstown series) adjoining a strip of woodland adjacent to the college experimental orchard. The complete chemical and mineralogical analyses of this soil have been reported elsewhere (**198**). Six inches of crushed limestone were placed at the bottom of each cylinder to prevent capillary rise of the surrounding soil into which the cylinders were sunk. The soil used in these cylinders was removed by layers and each horizon was thoroughly mixed during a period of many months before introduction into the cylinders in October, 1921. The total weight of soil used was 210 tons. This soil was then allowed to remain fallow for one year to permit the effects of the climatic factors on the physical, chemical, and microbiological conditions to become uniform in all cylinders.

2. PLANTING

In the spring of 1922, standard stocks vegetatively propagated from a single parent secured from the East Malling Agricultural Research Station, England, whip-grafted to Stayman Winesap scions, were planted. The scions were obtained from a tree whose performance was known for two bud generations (**3**). Growth measurements made at the end of the years 1922, 1923, and 1924 before fertilizer applications were made showed no significant differences between the trees in adjacent rows (**3**) (table II).

3. FERTILIZER TREATMENT

Nitrogen, phosphorus, and potassium were supplied in the form of C.P. salts, singly and also in the various combinations shown in the diagram (fig. 1). Analysis gave $NaNO_3$, 16.45 per cent. N; $CaH_4(PO_4)_2 \cdot 2H_2O$, 56.30 per cent. P_2O_5; and K_2SO_4, 53.40 per cent. K_2O. The following amounts per tree were added: $NaNO_3$, 918 gm.; K_2SO_4, 293 gm.; and $CaH_4(PO_4)_2 \cdot 2H_2O$, 534 gm., equivalent per acre of 100 trees to 35 lb. N, 35 lb. K_2O, and 70 lb. P_2O_5. If calculated in terms of percentage amounts present in the first 8 inches of surface soil (horizon A), the amounts added are equivalent to 0.0054 per cent. N, 0.0054 per cent. K_2O, and 0.0108 per cent. P_2O_5, respectively. These quantities of salts were not applied at one time; the first application was made in April, 1925, and the second on May 3, 1925.

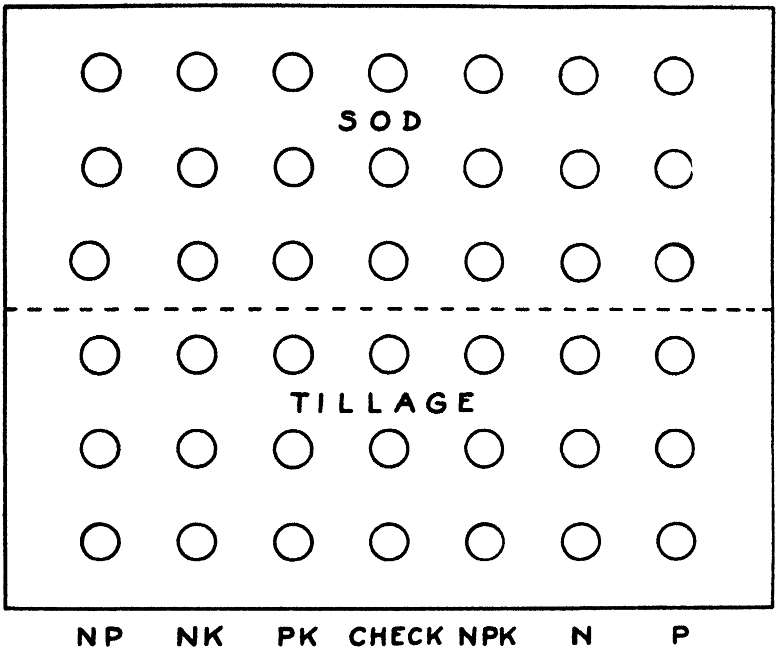

FIG. 1. Plan of experiment.

It should be noted that none of the trees bloomed before the treatment with fertilizers, *i.e.*, there were no blossoms in the spring of 1925 and, therefore, no fruit buds were formed in 1924. On May 12, 1926, the year following the fertilizer additions, the trees were deflorated, *i.e.*, the flower buds were removed just before the normal opening of flowers, to eliminate the influence of reproductive activities and fruit dominance on the growth and composition of the actively growing tissues (**9**, **127**, **128**).

4. Selection of samples for analysis

Inasmuch as this investigation was not concerned with the dynamics of the photosynthetic processes, but rather with the relationship of the internal changes produced by the principal nutrient elements to the vegetative and reproductive responses, it was decided in the first instance to examine the organs used for transport of food and for storage. The question then was, to what extent does the composition of the current and previous season's branch growth reflect the changes in metabolism which resulted in such striking differences in the growth of the trees in this experiment?

Kraybill, Potter, and Wentworth (**84**), Loomis (**100**), and also Potter and Phillips (**156**, **157**) suggest that the samples for analytical examination should be confined preferably to new spur growth, the spurs being nothing more than lateral branches which for some reason have made only limited growth. In the present experiment it was at the outset considered doubtful whether the spurs would provide sufficient material for the work planned. But even so there are sound reasons why investigations bearing on the internal factors associated with growth and fruit-bud formation should not be confined to the spurs alone.

A. REASONS FOR CHEMICAL EXAMINATION OF BRANCH GROWTH.—The chemical examination of the current and previous season's branch growth is of importance (**201**), for changes in the metabolic direction of the living cells of the woody stems must precede those in the developing bud. Hence, in the last analysis, conditions further back in the tree would *a priori* be expected to have considerable influence on the chemical system determining growth and fruiting. The investigations of **HOOKER** (**70**) and of **HOOKER** and BRADFORD (**72**), moreover, indicate that the variations in the chemical composition of bark taken from the scaffold limbs parallel closely those in the spurs, new growth, and last year's branch growth. And the writer (**201, 202**) has shown that, in so far as the nitrogen cycle is concerned, the seasonal variations in concentration of the various nitrogen fractions parallel one another in the current and previous season's branch growth.

B. DO INDIVIDUAL BRANCHES AND SPURS ACT AS A UNIT?—The principal difficulty in sampling fruit trees lies in obtaining homogeneous material without sensibly changing the nutritive conditions. If it could be shown that each spur acts as a unit, then errors arising from variability would cease to be much of a factor, even when only a few trees are available for analysis. Experimental and observational work exists that might seem to support the theory that individual branches and spurs act as a unit (**8, 105, 166, 226**). Thus, **AUCHTER** concluded from experiments on apple trees that mineral nutrients absorbed by the roots on one side of a plant are in large measure translocated to and used only by the trunk, limbs, and leaves directly above them; *i.e.*, there is no cross-over from one sector to another. But, as $NaNO_3$ was the only salt used in AUCHTER's experiments, and in view of the writer's results that nitrates are for the most part reduced in the fine roots of *Pyrus malus* (**203**), the possibility exists that AUCHTER's conclusions are valid only for *organic* materials (which move only in the phloem tissue) and not for the *mineral* (*i.e.*, ash) materials (**29, 111, 112, 113, 114, 115**). Neither the observations of **HARDY** (**57**) nor of MACK (**107**) support the view of the individuality of the spur. The latter found a uniformity of behavior of all spurs with respect to "off-year" bearing, "on-year" bearing, and "biennial" bearing.

These conflicting views may, perhaps, be reconciled when consideration is given to the fact that a fruit tree, although in effect a single organism, may be treated as a colony of competing units (**11, 62**). But even so the idea of independence can be carried too far, for a spur deprived of its own leaves can receive enough nutrient supply from adjacent parts of the tree to function actively (**62**). Moreover, the behavior of different varieties of fruit trees differs in this respect (**62**).

5. Collection of samples

On September 1, 1925, and succeeding dates (tables V to IX), samples of the current and previous season's branch growth were taken in the manner already described (**200, 201**) from the trees growing in the rims that were seeded to bluegrass on May 27, 1924, *i.e.*, from the trees growing in sod. In the following year (1926) samples were taken on April 4, June 21, August 15, and November 1. These periods may be characterized as follows: September 1, period of cessation of active growth; April 4, period of bud swelling; June 21–August 15, period of active growth; November 1, leaf fall and beginning of period of winter rest. As a matter of record, a summary of the meterological conditions is appended in table I.

TABLE I

Summary of meteorological data

Dates	Mean maximum	Mean minimum	Rainfall	Sunshine	Humidity
	° *F.*	° *F.*	*inches*	*per cent.*	*per cent.*
1925					
Aug. 15–Nov. 1	67.4	48.5	5.78		81.5
1926					
Apr. 4–June 30	69.2	43.4	7.34	70.4	72.5
June 30–Aug. 15	83.0	61.4	5.35	61.4	80.3
Aug. 15–Nov. 1	68.5	50.4	12.41	.	87.1

6. Separation of bark and wood

The bark (periderm, cortex, phloem) was separated from the wood (outer xylem, inner xylem) and each analyzed separately. In the present work no attempt was made to divide the complex constituents (tissues) of the bark and xylem, although it is realized that very limited regions may be involved in a particular response (**211, 215**).

Gradients.—There undoubtedly do exist slight differences in composition between the terminal, middle, and basal portions of the bark and wood, respectively, of shoots (**215**). But there is no evidence as yet of either a positive or negative gradient. Tufts (**215**) reported that differences in composition between the various portions so far as location up and down the shoot is concerned are relatively insignificant, compared with those existing between bark and wood in the same portions. Preliminary work to test this point carried out by the writer prior to the present studies showed very similar results.

In the usual macroscopic method of analysis, cells of admittedly widely different nature and function are taken: epidermal, vascular, and parenchymatous tissues are mixed indiscriminately. It is, therefore, only the predominant internal relations that are revealed by the methods adopted in the present study.

7. ANALYTICAL METHODS

A. DO THE VALUES IN TOTAL CARBON DETERMINATIONS REPRESENT POTENTIALLY MOBILE AND METABOLICALLY REACTIVE COMPOUNDS?—In the present investigation, fractionation of the nitrogen compounds similar to those previously carried out by the writer (**201, 202**) has not been undertaken. The possibilities and limitations of nitrogen partition investigations in this species have already been clearly defined (**202**). These earlier studies have shown, moreover, that the respective nitrogen fractions were measurable functions of the total nitrogen, even under widely differing external environments. Thus, the total water-soluble N, the non-protein N, and the amino N closely paralleled the total N throughout the growth cycle; the amide N and "rest" N varied inversely with them. It would seem, therefore, that amino N must be connected with protein synthesis and "rest" N with protein degradation. Accordingly, since the sequences of variations (changes) in the distribution of partially synthesized proteins parallel the total N, the seasonal fluctuations of the former may be predicted from the time-concentration curves of the latter.

On the other hand, if any definite relationships exist between the total carbohydrate and its various fractions, they have not been identified in any metabolism studies thus far carried out. It was considered desirable, therefore, to undertake the determination of such carbohydrate fractions as are amenable to present methods of analysis rather than the determination of total carbon preferred by some investigators (**63**), who consider that the determination of total carbon is sufficient to establish the relationship existing between the carbon and nitrogen compounds present. HICKS (**63**) justifies the determination of total carbon, without fractionation according to the usual methods, on the basis that the primary value of the C/N relation lies in the fact that the growth of a plant is dependent upon the balance between the metabolic processes of carbon assimilation and respiration on the one hand and absorption of nitrogen from the soil on the other; and that, since both processes involve the use of "raw" inorganic material in the form of carbon dioxide and nitrates, the relation of real importance in the plant itself, according to her view, is that of elemental carbon to elemental nitrogen. Although this investigator recognizes the fact that part of the assimilated carbon is transformed into immobile compounds, such as lignin, she maintains that these immobile compounds have not been shown to lie outside the vital system, and, therefore, to be without influence on the plant. It would appear that the problem of the plant physiologist is not so simple in this respect as that of the animal physiologist (**81**). Nevertheless, *the burden of proof remains with the proponents of the "total" carbon idea to show that the carbon compounds of plants are as reactive and mobile as the nitrogen compounds with which they are compared.*

B. WATER.—(1) The imbibitional water was determined by drying the material in a drying oven at a temperature of 70°–80°. The samples were afterwards left exposed to the air for several weeks and weighed when equilibrium had been reached. (2) The hygroscopic water was determined by heating 2 grams of the finely ground materials *in vacuo* at 100°.

C. ASH.—Ten grams of the finely ground material were ignited in an electric muffle furnace at a temperature not exceeding 450° to avoid loss of potassium salts.

D. POTASSIUM.—The usual LINDO-GLADDEN method was employed (**7**).

E. PHOSPHORUS.—The phosphorus was precipitated as the ammonium phospho-molybdate after the method of STEWART (**186**) and the precipitate was titrated immediately according to RICHARDS and GODDEN's modification (**164**) of the PEMBERTON-NEUMANN method (**133, 151**).

F. NITROGEN.—Nitrogen was determined by the usual KJELDAHL method (**7**). Only traces of nitrates are present in apple tissues (**38, 203**).

G. CARBOHYDRATES.—The estimation of the initial separation of carbohydrates was effected by the method proposed by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists into a "soluble" and an "insoluble" fraction (**5**). In the "soluble" portion: (1) reducing sugars (glucose, fructose, pentose, arabinose, and xylose) and (2) non-reducing sugars (sucrose and other disaccharides) were determined. Glucosides such as phloridzin (**61**) were not separately estimated; these compounds would be included in the latter group.

It is of interest to note that it has recently been questioned if phloridzin is a true glucoside. MOELWYN-HUGHES (**123**) finds that phloridzin is not hydrolyzed by emulsin but by another enzyme occurring freely in nature—probably β–(γ)-fructosidase. Moreover, he also points out that all the published data on the velocity of hydrolysis of glucosides by acids indicate that phloridzin resembles, with respect to velocity coefficient and critical increment for hydrolysis, the γ-fructosides (sucrose, raffinose, melezitose) more closely than the normal glucosides (salicin, arbutin, maltose, etc.).

The determinations of the various fractions were made according to the writer's method (**199**). The acid-hydrolyzable "insoluble" fractions (**4**, **5**) include the ill-defined groups designated by SCHULZE (**172**) as "hemicellulose," and which may function as reserve material (**129, 167**). In the present investigation the term includes hexosans, pentosans, and such mixed polymers as pectins and gums.

III. Presentation of results

1. GROWTH MEASUREMENTS

Throughout the period 1922–24 inclusive, *i.e.,* before the nutrient salts were added, there was no significant difference in growth measurements in trees from different rows (**3**) (table III). The response of the trees to nitrogen additions was especially striking. Within six weeks after the first application in the spring of 1925, the leaves of the trees receiving nitrogen additions changed from a pale green to a deep green color. At the end of

TABLE II

EFFECT OF TREATMENTS ON GROWTH OF GRASS CUT IN 1925

TREATMENT	WEIGHT OF GRASS CLIPPINGS
	gm.
Check	845
NPK	5708
NP	5365
NK	2673
PK	1760
N	3743
P	1457

TABLE III

BRANCH ELONGATION IN CENTIMETERS OF SEASON'S BRANCH GROWTH (1922–1926)

TREATMENT (AFTER 1924)	SOD				
	1922	1923	1924	1925*	1926
Check	851	3270	5005	3051	985
NPK	863	2560	4662	6991	6578
NP	429	2637	4983	6475	7795
NK	648	3402	5094	5808	5031
PK	762	2517	4881	3247	2073
N	570	2796	3844	5564	5243
P	635	2696	4537	3247	1790

* First year of fertilizer application.

this period marked differences in growth responses were observed. The soil condition with respect to supply of nutrients at this time is indicated by the combined weight of the grass clippings (table II), taken in June, 1925, from the "sod" cylinders and also of the combined branch elongation at the end of the 1925 growing season (**3**) (table III) for all three trees in each treatment.

The effect of the differential fertilizer treatments given in the spring of 1925 on the fruit bud formation of that year is shown in table IV, which gives the number of blossom clusters removed from the three trees in each treatment in 1926 (**3**) when the trees were deflorated.

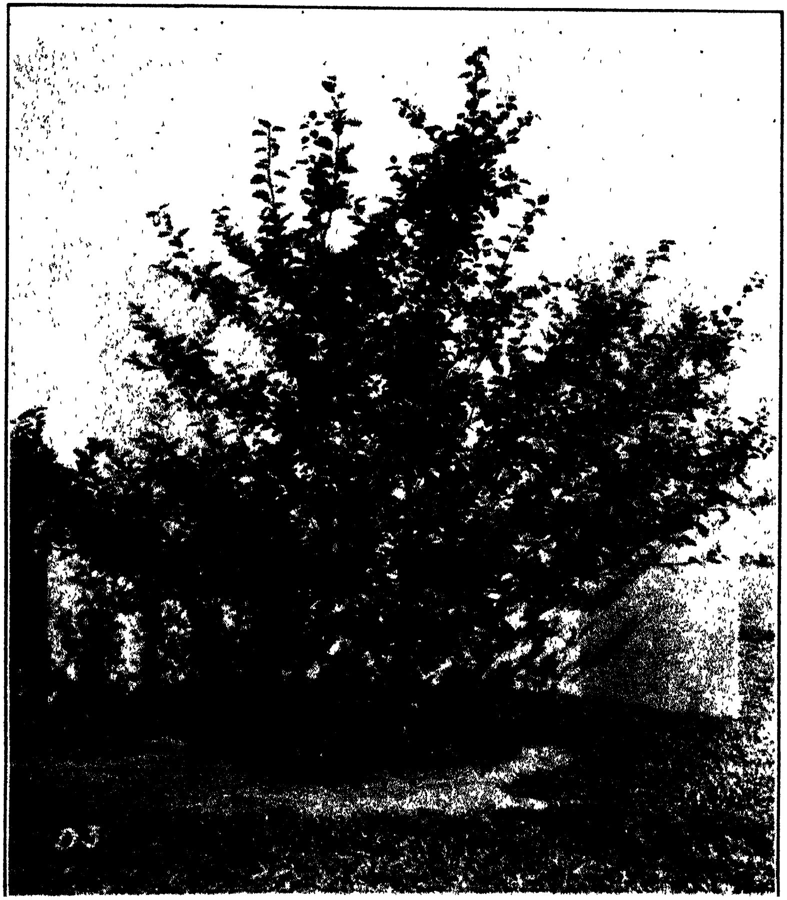

Fig. 2. Typical tree growing in sod treated with the three nutrient salts: $NaNO_3$, K_2SO_4, and $CaH_4(PO_4)_2 \cdot 2H_2O$.

TABLE IV

EFFECT OF TREATMENTS ON NUMBER OF BLOSSOM CLUSTERS IN 1926

TREATMENT	SOD	CULTIVATED
Check	0	6
NPK	328	353
NP	145	334
NK	102	17
PK	0	28
N	21	71
P	0	13

It may be pointed out here that the tips of the shoots of the check trees and also of those receiving only phosphorus had relatively little active cambium compared with the trees of other treatments. The nitrogen-treated trees were differentiated from the others in having less xylem with thinner walls than the trees without nitrogen additions. The cambium, too, of the trees receiving added nitrogen was active even to the base of the stem.

FIG. 3. Typical tree growing in sod without nutrient salt additions.

Figure 2 shows a representative tree from the row in sod and receiving all three of the nutritive elements nitrogen, phosphorus, and potassium. Figure 3 shows a representative check tree receiving no nutrient additions.

2. ANALYTICAL DATA

In the following tables the term "glucose" will be used to designate the reducing sugars estimated as glucose (p. 401), and the term "sucrose" to indicate the non-reducing sugars and glucosides (p. 401). The analytical results are presented in tables V–IX.

TABLE V

PERCENTAGES OF WATER IN SEASON'S AND IN ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	IMBIBITIONAL WATER		HYGROSCOPIC WATER		TOTAL WATER	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	39.42	47.00	2.23	2.72	41.65	49.72
58	59	Apr. 4, 1926		42.08	51.60	2.06	2.80	44.14	54.40
89A	89B	June 30, "		40.70	50.16	1.80	2.55	42.50	52.71
98	99	Aug. 15, "		38.80	46.70	1.40	2.70	40.20	49.40
185	186	Nov. 1, "		37.24	45.05	1.46	2.30	38.70	47.35
9	10	Sept. 1, 1925	NPK	43.11	53.70	2.54	4.05	45.65	57.75
50	51	Apr. 4, 1926		45.68	55.60	2.72	3.96	48.40	59.56
90A	90B	June 30, "		45.93	54.60	2.32	4.20	48.25	58.80
94	95	Aug. 15, "		44.10	52.90	2.40	4.80	46.58	57.70
182	183	Nov. 1, "		40.60	48.70	2.80	3.85	43.40	52.55
1	2	Sept. 1, 1925	NP	43.97	50.60	2.75	3.85	46.72	54.45
82	83	Apr. 4, 1926		44.25	55.00	2.72	3.57	46.97	58.57
85A	85B	June 30, "		45.15	52.30	2.35	3.90	47.50	56.20
110	111	Aug. 15, "		44.43	50.10	2.10	3.80	46.53	53.90
194	195	Nov. 1, "		38.70	47.60	2.02	4.00	40.72	51.60
3	4	Sept. 1, 1925	NK	42.50	50.12	2.60	3.60	45.10	53.72
74	75	Apr. 4, 1926		44.36	54.80	2.52	3.30	46.88	58.10
86A	86B	June 30, "		44.22	51.45	2.38	3.75	46.60	55.20
106	107	Aug. 15, "		43.07	48.20	2.15	3.80	45.22	52.06
191	192	Nov. 1, "		38.04	50.50	2.06	3.90	44.10	50.30
5	6	Sept. 1, 1925	PK	43.38	50.27	2.50	3.56	45.88	53.83
66	67	Apr. 4, 1926		44.26	54.70	2.08	3.76	46.34	58.46
88A	88B	June 30, "		43.10	50.86	2.30	3.85	45.50	54.71
102	103	Aug. 15, "		41.60	49.30	2.60	4.00	44.20	53.30
188	189	Nov. 1, "		40.65	49.40	2.35	3.50	43.00	52.90
11	12	Sept. 1, 1925	N	42.10	52.20	2.40	4.00	44.50	56.20
42	43	Apr. 4, 1926		42.50	55.40	2.78	3.57	45.28	58.97
91A	91B	June 30, "		44.08	54.05	2.15	4.15	46.23	58.20
95	96	Aug. 15, "		42.20	51.73	2.30	3.90	44.50	55.63
179	180	Nov. 1, "		40.50	47.80	2.25	4.00	42.75	51.80
13	14	Sept. 1, 1925	P	38.95	47.13	2.25	2.72	41.20	49.85
34	35	Apr. 4, 1926		42.38	51.10	2.20	2.65	44.58	53.75
92A	92B	June 30, "		41.28	50.18	1.72	2.40	43.00	52.58
97	98	Aug. 15, "		38.40	47.10	2.00	2.80	40.40	49.90
176	177	Nov. 1, "		37.55	46.45	1.65	2.65	39.20	49.10

TABLE VI

AMOUNTS OF GLUCOSE, SUCROSE, TOTAL SUGARS, STARCH, HEMICELLULOSE, AND "TOTAL" AVAILABLE CARBOHYDRATES AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF FRESH WEIGHT OF MATERIAL											
				GLUCOSE		SUCROSE		TOTAL SUGARS		STARCH		HEMICELLULOSE		TOTAL AVAILABLE CARBOHYDRATES	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	0.117	0.292	0.140	0.503	0.257	0.794	5.946	5.742	18.042	10.483	24.244	17.019
58	59	Apr. 4, 1926		0.229	0.401	0.078	0.410	0.307	0.812	6.173	5.016	16.021	9.758	22.500	15.586
89A	89B	June 30, "		0.104	0.189	0.035	0.355	0.138	0.544	4.859	5.490	17.290	10.427	22.287	16.462
98	99	Aug. 15, "		0.167	0.278	0.114	0.430	0.281	0.708	5.532	6.527	18.299	11.132	24.111	18.368
185	186	Nov. 1, "		0.337	0.400	0.153	0.316	0.490	0.716	6.743	6.349	17.783	11.583	25.017	18.649
9	10	Sept. 1, 1925	NPK	0.217	1.014	0.228	0.668	0.446	1.682	4.033	3.046	13.756	7.977	18.234	12.705
50	51	Apr. 4, 1926		0.325	0.768	0.175	0.384	0.501	1.153	4.051	1.415	11.796	7.869	16.347	10.439
90A	90B	June 30, "		0.181	0.692	0.202	0.124	0.383	0.816	1.656	2.493	11.519	8.446	13.559	11.754
94	95	Aug. 15, "		0.241	0.973	0.257	0.275	0.498	1.248	2.408	3.215	13.857	8.058	16.227	12.521
182	183	Nov. 1, "		0.464	1.205	0.255	0.664	0.719	1.869	4.415	2.159	13.324	10.425	18.457	14.453
1	2	Sept. 1, 1925	NP	0.234	1.098	0.202	0.706	0.437	1.804	4.529	3.553	12.749	8.126	17.716	13.483
82	83	Apr. 4, 1926		0.286	0.766	0.159	0.423	0.445	1.189	4.667	1.595	11.858	8.526	16.969	11.310
85A	85B	June 30, "		0.210	0.701	0.184	0.197	0.394	0.898	1.890	2.602	12.731	8.961	14.700	12.461
110	111	Aug. 15, "		0.257	1.014	0.267	0.401	0.524	1.415	2.940	3.467	12.908	8.759	16.383	13.650
194	195	Nov. 1, "		0.462	1.210	0.308	0.750	0.178	1.960	5.068	2.688	12.751	10.096	18.590	14.743
3	4	Sept. 1, 1925	NK	0.220	1.110	0.192	0.671	0.412	1.781	4.914	4.128	13.516	8.502	18.842	14.412
74	75	Apr. 4, 1926		0.297	0.859	0.133	0.453	0.430	1.311	4.249	2.263	12.499	8.988	17.179	12.562
86A	86B	June 30, "		0.203	0.717	0.235	0.215	0.438	0.932	2.429	3.069	11.983	10.022	14.851	14.022
106	107	Aug. 15, "		0.301	0.791	0.247	0.431	0.548	1.222	3.424	3.452	12.489	10.810	16.461	14.526
191	192	Nov. 1, "		0.425	1.093	0.280	0.820	0.704	1.913	3.913	2.922	13.382	11.814	17.999	15.656
5	6	Sept. 1, 1925	PK	0.162	0.254	0.179	0.323	0.341	0.577	4.654	4.178	15.522	10.125	20.517	14.881
66	67	Apr. 4, 1926		0.279	0.415	0.145	0.291	0.424	0.706	4.937	3.431	15.339	9.329	18.711	13.459
88A	88B	June 30, "		0.137	0.453	0.087	0.272	0.224	0.725	4.368	4.022	13.224	10.426	17.816	15.172
102	103	Aug. 15, "		0.206	0.318	0.156	0.294	0.363	0.612	4.788	4.483	15.507	10.839	20.657	15.934
188	189	Nov. 1, "		0.371	0.471	0.199	0.471	0.570	0.942	5.529	4.823	13.999	10.367	20.098	16.132
11	12	Sept. 1, 1925	N	0.244	0.876	0.194	0.701	0.438	1.577	4.190	4.117	15.351	8.725	19.980	14.419
42	43	Apr. 4, 1926		0.317	0.698	0.148	0.492	0.465	1.190	4.514	2.975	12.870	7.898	17.849	12.063
91A	91B	June 30, "		0.161	0.640	0.231	0.418	0.393	1.058	2.581	2.926	12.754	8.819	15.728	12.803
95	96	Aug. 15, "		0.233	0.799	0.250	0.541	0.483	1.340	3.441	3.727	14.114	9.096	18.038	14.163
179	180	Nov. 1, "		0.451	0.988	0.315	0.819	0.767	1.808	4.866	4.299	14.232	9.568	19.866	15.675
13	14	Sept. 1, 1925	P	0.129	0.376	0.112	0.707	0.241	1.083	5.733	5.667	17.910	10.105	23.885	16.855
34	35	Apr. 4, 1926		0.211	0.347	0.155	0.324	0.366	0.671	5.658	5.134	15.396	9.689	21.419	15.494
92A	92B	June 30, "		0.086	0.190	0.114	0.488	0.199	0.678	4.138	4.837	16.781	10.243	21.119	15.758
97	98	Aug. 15, "		0.161	0.301	0.131	0.326	0.292	0.651	4.765	5.882	18.363	10.341	23.423	17.149
176	177	Nov. 1, "		0.334	0.356	0.134	0.255	0.468	0.611	6.749	6.067	16.708	10.638	23.925	17.316

TABLE VI—(*Continued*)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF DRY WEIGHT OF MATERIAL											
				GLUCOSE		SUCROSE		TOTAL SUGARS		STARCH		HEMICELLULOSE		TOTAL AVAILABLE CARBOHYDRATES	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	0.20	0.58	0.24	1.00	0.44	1.58	10.19	11.42	30.92	20.85	41.55	33.85
58	59	Apr. 4, 1926		0.41	0.88	0.14	0.90	0.55	1.78	11.05	11.00	28.68	21.40	40.28	34.18
89A	89B	June 30, "		0.18	0.40	0.06	0.75	0.24	1.15	8.45	11.61	30.07	22.05	38.76	34.81
98	99	Aug. 15, "		0.28	0.55	0.19	0.85	0.47	1.40	9.25	12.90	30.60	22.00	40.32	36.30
185	186	Nov. 1, "		0.55	0.76	0.25	0.60	0.80	1.36	11.00	12.06	29.01	22.00	40.81	35.42
9	10	Sept. 1, 1925	NPK	0.40	2.40	0.42	1.58	0.82	3.98	7.42	7.21	25.31	18.88	33.55	30.07
50	51	Apr. 4, 1926		0.63	1.90	0.34	0.95	0.97	2.85	7.85	3.50	22.86	19.46	31.68	25.81
90A	90B	June 30, "		0.35	1.68	0.39	0.30	0.74	1.98	3.20	6.05	22.26	20.50	26.20	28.63
94	95	Aug. 15, "		0.45	2.30	0.48	0.65	0.93	2.95	4.50	7.60	25.90	19.05	30.33	29.60
182	183	Nov. 1, "		0.82	2.54	0.45	1.40	1.27	3.94	7.80	4.55	23.54	21.97	32.61	30.46
1	2	Sept. 1, 1925	NP	0.44	2.41	0.38	1.55	0.82	3.96	8.50	7.80	23.93	17.84	33.25	29.60
82	83	Apr. 4, 1926		0.54	1.85	0.30	1.02	0.84	2.87	8.80	3.85	22.36	20.58	32.00	27.30
85A	85B	June 30, "		0.40	1.60	0.35	0.45	0.75	2.05	3.60	5.94	24.25	20.46	28.00	28.45
110	111	Aug. 15, "		0.48	2.20	0.50	0.87	0.98	3.07	5.50	7.52	24.14	19.00	30.64	29.61
194	195	Nov. 1, "		0.78	2.50	0.52	1.55	1.30	4.05	8.55	5.55	21.51	20.86	31.36	30.46
3	4	Sept. 1, 1925	NK	0.40	2.40	0.35	1.45	0.75	3.85	8.95	8.92	24.62	18.37	34.32	31.14
74	75	Apr. 4, 1926		0.56	2.05	0.25	1.08	0.81	3.13	8.00	5.40	23.53	21.45	32.34	29.98
86A	86B	June 30, "		0.38	1.60	0.44	0.48	0.82	2.08	4.55	6.85	22.44	22.37	27.81	31.30
106	107	Aug. 15, "		0.55	1.65	0.45	0.90	1.00	2.55	6.25	7.20	22.80	22.55	30.05	30.30
191	192	Nov. 1, "		0.76	2.20	0.50	1.65	1.26	3.85	7.00	5.88	23.94	23.77	32.20	31.50
5	6	Sept. 1, 1925	PK	0.30	0.55	0.33	0.70	0.63	1.25	8.60	9.05	28.68	21.93	37.91	32.23
66	67	Apr. 4, 1926		0.52	1.00	0.27	0.70	0.79	1.70	9.20	8.26	24.86	22.46	34.85	32.40
88A	88B	June 30, "		0.25	1.00	0.16	0.60	0.41	1.60	8.00	8.88	24.22	23.02	32.63	33.50
102	103	Aug. 15, "		0.37	0.68	0.28	0.63	0.65	1.31	8.58	9.60	27.79	23.21	37.02	34.12
188	189	Nov. 1, "		0.65	1.00	0.35	1.00	1.00	2.00	9.70	10.24	24.56	22.01	35.26	34.25
11	12	Sept. 1, 1925	N	0.44	2.00	0.35	1.60	0.79	3.60	7.55	9.40	27.66	19.92	36.00	32.92
42	43	Apr. 4, 1926		0.58	1.70	0.27	1.20	0.85	2.90	8.25	7.25	23.52	19.25	32.62	29.40
91A	91B	June 30, "		0.30	1.53	0.43	1.00	0.73	2.53	4.80	7.00	23.72	21.10	29.25	30.63
95	96	Aug. 15, "		0.42	1.80	0.45	1.22	0.87	3.02	6.20	8.40	25.43	20.50	32.50	31.92
179	180	Nov. 1, "		0.79	2.05	0.55	1.70	1.34	3.75	8.50	8.02	24.86	19.85	34.70	32.52
13	14	Sept. 1, 1925	P	0.22	0.75	0.19	1.41	0.41	2.16	9.75	11.30	30.46	20.15	40.62	33.61
34	35	Apr. 4, 1926		0.38	0.75	0.28	0.70	0.66	1.45	10.21	11.10	27.78	20.95	38.65	33.50
92A	92B	June 30, "		0.15	0.40	0.20	1.03	0.35	1.43	7.26	10.20	29.44	21.60	37.05	33.23
97	98	Aug. 15, "		0.27	0.60	0.22	0.65	0.49	1.30	8.00	11.74	30.81	20.64	39.30	34.23
176	177	Nov. 1, "		0.55	0.70	0.22	0.50	0.77	1.20	11.10	11.92	27.48	20.90	39.35	34.02

TABLE VII

AMOUNT OF ASH, NITROGEN, K_2O, AND P_2O_5 IN FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF FRESH WEIGHT OF MATERIAL							
				ASH		NITROGEN		K_2O		P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	0.957	3.469	0.088	0.327	0.108	0.346	0.118	0.129
58	59	Apr. 4, 1926		0.754	2.868	0.152	0.424	0.106	0.319	0.103	0.091
89A	89B	June 30, "		0.661	2.781	0.166	0.364	0.129	0.369	0.079	0.097
98	99	Aug. 15, "		0.927	3.087	0.111	0.364	0.119	0.379	0.102	0.116
185	186	Nov. 1, "		1.017	3.596	0.155	0.432	0.116	0.411	0.106	0.111
9	10	Sept. 1, 1925	NPK	0.935	3.207	0.261	0.393	0.136	0.404	0.136	0.129
50	51	Apr. 4, 1926		0.877	2.588	0.397	0.582	0.137	0.549	0.116	0.104
90A	90B	June 30, "		0.828	2.472	0.422	0.478	0.145	0.476	0.099	0.108
94	95	Aug. 15, "		0.899	2.939	0.321	0.474	0.134	0.431	0.128	0.149
182	183	Nov. 1, "		1.030	3.748	0.351	0.702	0.169	0.607	0.167	0.185
1	2	Sept. 1, 1925	NP	0.938	3.316	0.267	0.409	0.109	0.323	0.132	0.132
82	83	Apr. 4, 1926		0.875	2.631	0.379	0.539	0.125	0.348	0.117	0.104
85A	85B	June 30, "		0.840	2.694	0.409	0.517	0.123	0.398	0.092	0.110
110	111	Aug. 15, "		0.866	2.914	0.353	0.507	0.134	0.419	0.126	0.161
194	195	Nov. 1, "		1.186	3.520	0.412	0.600	0.169	0.465	0.178	0.186
3	4	Sept. 1, 1925	NK	0.961	3.332	0.253	0.213	0.118	0.421	0.109	0.123
74	75	Apr. 4, 1926		0.797	2.614	0.339	0.267	0.119	0.417	0.099	0.092
86A	86B	June 30, "		0.812	2.710	0.350	0.294	0.149	0.576	0.078	0.101
106	107	Aug. 15, "		0.887	2.968	0.264	0.231	0.131	0.489	0.108	0.127
191	192	Nov. 1, "		0.950	3.648	0.313	0.278	0.162	0.571	0.113	0.142
5	6	Sept. 1, 1925	PK	0.963	3.232	0.203	0.360	0.124	0.332	0.128	0.132
66	67	Apr. 4, 1926		0.789	2.575	0.280	0.407	0.131	0.353	0.119	0.102
88A	88B	June 30, "		0.699	2.649	0.290	0.403	0.145	0.451	0.079	0.112
102	103	Aug. 15, "		0.809	2.956	0.246	0.364	0.106	0.411	0.103	0.124
188	189	Nov. 1, "		0.912	3.249	0.286	0.419	0.145	0.436	0.117	0.134
11	12	Sept. 1, 1925	N	0.932	3.053	0.244	0.394		0.311	0.126	0.125
42	43	Apr. 4, 1926		0.821	2.585	0.375	0.501	0.120	0.304	0.111	0.099
91A	91B	June 30, "		0.758	2.541	0.409	0.422	...	0.378	0.076	0.100
95	96	Aug. 15, "		0.888	2.817	0.297	0.430	0.128	0.346	0.111	0.106
179	180	Nov. 1, "		0.967	3.441	0.313	0.511	0.146	0.395	0.119	0.125
13	14	Sept. 1, 1925	P	0.929	3.480	0.106	0.376	..	0.341	0.126	0.130
34	35	Apr. 4, 1926		0.798	2.775	0.202	0.402	0.102	0.333	0.116	0.093
92A	92B	June 30, "		0.684	2.802	0.205	0.379		0.382	0.083	0.099
97	98	Aug. 15, "		0.924	3.131	0.163	0.376	0.107	0.371	0.113	0.116
176	177	Nov. 1, "		1.046	3.507	0.182	0.407	0.106	0.412	0.131	0.109

TABLE VII—(*Continued*)

AMOUNT OF ASH, NITROGEN, K_2O, AND P_2O_5 IN FRESH AND DRY WEIGHTS, RESPECTIVELY, OF THE SEASON'S AND ONE-YEAR BRANCH GROWTH (WOOD AND BARK SEPARATED)

LABORATORY NO.		SAMPLING DATE	TREATMENT	PERCENTAGES OF DRY WEIGHT OF MATERIAL							
				ASH		NITROGEN		K_2O		P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	1.64	6.90	0.151	0.65	0.185	0.689	0.203	0.256
58	59	Apr. 4, 1926		1.35	6.29	0.273	0.93	0.190	0.700	0.185	0.200
89A	89B	June 30, "		1.15	5.88	0.288	0.77	0.225	0.780	0.138	0.205
98	99	Aug. 15, "		1.55	6.10	0.185	0.72	0.200	0.750	0.170	0.230
185	186	Nov. 1, "		1.66	6.83	0.253	0.82	0.190	0.780	0.173	0.210
9	10	Sept. 1, 1925	NPK	1.72	7.59	0.480	0.93	0.250	0.957	0.250	0.306
50	51	Apr. 4, 1926		1.70	6.40	0.770	1.44	0.265	1.020	0.225	0.258
90A	90B	June 30, "		1.60	6.00	0.815	1.16	0.280	1.360	0.191	0.262
94	95	Aug. 15, "		1.68	6.95	0.600	1.12	0.250	1.155	0.240	0.354
182	183	Nov. 1, "		1.82	7.90	0.620	1.48	0.300	1.280	0.295	0.390
1	2	Sept. 1, 1925	NP	1.76	7.28	0.502	0.90	0.205	0.710	0.248	0.290
82	83	Apr. 4, 1926		1.65	6.35	0.715	1.30	0.235	0.840	0.220	0.250
85A	85B	June 30, "		1.60	6.15	0.780	1.18	0.235	0.910	0.175	0.252
110	111	Aug. 15, "		1.62	6.32	0.660	1.10	0.250	0.910	0.235	0.350
194	195	Nov. 1, "		2.00	7.48	0.695	1.24	0.285	0.960	0.300	0.385
3	4	Sept. 1, 1925	NK	1.75	7.20	0.460	0.89	0.215	0.910	0.200	0.265
74	75	Apr. 4, 1926		1.50	6.24	0.638	1.29	0.225	0.995	0.188	0.220
86A	86B	June 30, "		1.52	6.05	0.656	1.12	0.280	1.285	0.146	0.225
106	107	Aug. 15, "		1.62	6.15	0.482	1.08	0.240	1.022	0.198	0.265
191	192	Nov. 1, "		1.70	7.34	0.560	1.22	0.290	1.150	0.203	0.285
5	6	Sept. 1, 1925	PK	1.78	7.00	0.375	0.78	0.230	0.720	0.237	0.285
66	67	Apr. 4, 1926		1.47	6.20	0.522	0.98	0.245	0.850	0.222	0.245
88A	88B	June 30, "		1.28	5.85	0.532	0.89	0.265	0.995	0.144	0.242
102	103	Aug. 15, "		1.45	6.33	0.440	0.78	0.260	0.880	0.185	0.266
188	189	Nov. 1, "		1.60	6.90	0.502	0.89	0.275	0.965	0.206	0.285
11	12	Sept. 1, 1925	N	1.68	6.97	0.440	0.90	....	0.710	0.227	0.285
42	43	Apr. 4, 1926		1.50	6.30	0.685	1.22	0.220	0.740	0.202	0.241
91A	91B	June 30, "		1.41	6.08	0.762	1.01		0.905	0.141	0.240
95	96	Aug. 15, "		1.60	6.35	0.535	0.97	0.230	0.780	0.200	0.240
179	180	Nov. 1, "		1.69	7.14	0.546	1.06	0.255	0.820	0.208	0.260
13	14	Sept. 1, 1925	P	1.58	6.94	0.180	0.75		0.680	0.215	0.260
34	35	Apr. 4, 1926		1.44	6.00	0.365	0.87	0.185	0.720	0.210	0.200
92A	92B	June 30, "		1.20	5.91	0.360	0.80		0.805	0.145	0.210
97	98	Aug. 15, "		1.55	6.25	0.273	0.75	0.180	0.740	0.190	0.232
176	177	Nov. 1, "		1.72	6.89	0.300	0.80	0.175	0.810	0.216	0.215

TABLE VIII

RATIOS OF CARBOHYDRATE FRACTIONS, AND ALSO OF PHOSPHORIC ACID AND POTASH TO NITROGEN, CALCULATED AS PERCENTAGES OF DRY WEIGHT OF MATERIAL

LABORATORY NO.		SAMPLING DATE	TREATMENT	GLUCOSE/N		SUCROSE/N		STARCH/N		AVAILABLE CARBOHYDRATES/N		N/P_2O_5		K_2O/N	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	1.33	0.89	1.59	1.54	67.48	17.57	275.16	52.0	0.74	2.54	1.225	1.060
58	59	Apr. 4, 1926		1.50	0.95	0.51	0.97	40.48	11.83	147.55	35.8	2.48	4.65	0.696	0.753
89A	89B	June 30, "		0.63	0.52	0.21	0.97	29.34	15.06	134.58	45.2	2.09	3.76	0.781	1.013
98	99	Aug. 15, "		1.51	0.76	1.03	1.18	50.00	17.79	217.95	48.9	1.09	3.15	1.081	1.034
185	186	Nov. 1, "		2.17	0.93	0.99	0.73	43.48	14.68	161.30	43.1	1.46	3.90	0.751	0.951
9	10	Sept. 1, 1925	NPK	0.83	2.58	0.88	1.69	15.46	7.75	69.89	31.6	0.94	3.04	0.521	1.029
50	51	Apr. 4, 1926		0.82	1.32	0.44	0.68	10.19	2.43	41.14	18.6	2.64	5.58	0.344	0.708
90A	90B	June 30, "		0.43	1.45	0.48	0.26	3.63	5.22	32.15	24.0	3.48	4.49	0.343	1.172
94	95	Aug. 15, "		0.75	2.05	0.80	0.58	7.50	6.79	52.22	26.4	1.50	3.16	0.417	1.031
182	183	Nov. 1, "		1.32	1.72	0.73	0.95	12.08	3.07	52.59	20.5	1.30	3.79	0.484	0.865
1	2	Sept. 1, 1925	NP	0.88	2.68	0.76	1.72	16.93	8.67	66.23	32.9	2.02	3.10	0.408	0.789
82	83	Apr. 4, 1926		0.76	1.42	0.42	0.78	12.31	2.96	44.76	21.0	3.25	5.00	0.329	0.569
85A	85B	June 30, "		0.51	1.36	0.45	0.38	3.85	5.63	35.90	24.1	4.46	4.14	0.301	0.784
110	111	Aug. 15, "		0.73	2.00	0.76	0.79	8.33	6.84	46.42	26.9	2.81	3.10	0.333	0.709
194	195	Nov. 1, "		1.12	2.02	0.75	1.17	12.30	4.48	45.12	24.5	2.32	3.22	0.410	0.661
3	4	Sept. 1, 1925	NK	0.87	2.70	0.76	1.63	19.46	9.91	74.61	34.5	2.30	3.40	0.576	1.011
74	75	Apr. 4, 1926		0.88	1.59	0.39	0.84	12.54	4.19	50.69	23.2	3.39	5.86	0.446	0.771
86A	86B	June 30, "		0.58	1.43	0.67	0.43	6.94	6.12	42.39	27.9	4.49	4.98	0.426	1.147
106	107	Aug. 15, "		1.14	1.53	0.93	0.83	12.96	6.67	62.34	29.9	2.43	4.08	0.496	0.946
191	192	Nov. 1, "		1.36	1.80	0.89	1.35	12.50	4.82	57.50	27.4	2.76	4.28	0.518	0.943
5	6	Sept. 1, 1925	PK	0.80	0.71	0.88	0.89	22.93	11.60	101.09	41.3	1.57	2.74	0.613	0.923
66	67	Apr. 4, 1926		0.99	1.02	0.52	0.71	17.62	8.43	66.76	32.4	2.35	4.00	0.469	0.867
88A	88B	June 30, "		0.47	1.12	0.30	0.67	15.04	9.98	61.33	37.6	3.69	3.79	0.498	1.118
102	103	Aug. 15, "		0.84	0.87	0.64	0.81	19.50	12.31	84.14	43.8	2.38	3.00	0.432	1.129
188	189	Nov. 1, "		1.29	1.12	0.69	1.12	19.32	11.51	70.23	38.4	2.44	3.12	0.508	1.039
11	12	Sept. 1, 1925	N	1.00	2.22	0.79	1.78	17.16	10.44	81.82	36.5	0.85	3.16		0.794
42	43	Apr. 4, 1926		0.85	1.39	0.39	0.98	12.04	5.94	47.62	24.1	2.32	5.06	0.321	0.689
91A	91B	June 30, "		0.39	1.51	0.56	0.99	6.30	6.90	38.39	30.1	4.12	4.23		0.872
95	96	Aug. 15, "		0.79	1.86	0.84	1.26	11.59	8.66	60.75	32.9	1.43	4.04	0.430	0.938
179	180	Nov. 1, "		1.45	1.93	1.01	1.60	15.57	8.39	63.55	30.6	1.43	4.08	0.467	0.906
13	14	Sept. 1, 1925	P	1.22	1.00	1.06	1.88	54.17	15.07	225.67	44.8	0.15	2.88		0.907
34	35	Apr. 4, 1926		1.04	0.86	0.77	0.80	27.97	12.76	105.89	38.5	0.63	4.35	0.507	0.828
92A	92B	June 30, "		0.42	0.50	0.56	1.29	20.17	12.75	102.92	41.5	0.89	3.81		0.944
97	98	Aug. 15, "		0.99	0.80	0.81	0.87	29.30	15.60	143.96	45.6	0.39	3.57	0.659	0.860
176	177	Nov. 1, "		1.83	0.88	0.73	0.63	37.00	14.87	131.17	42.5	0.42	4.00	0.583	1.013

TABLE IX

RATIOS OF CARBOHYDRATE FRACTIONS TO PHOSPHORIC ACID AND POTASH, CALCULATED AS PERCENTAGES OF DRY WEIGHT OF MATERIAL

LABORATORY NO.		SAMPLING DATE	TREATMENT	STARCH/K_2O		STARCH/P_2O_5		CARBOHYDRATE/K_2O		CARBOHYDRATE/P_2O_5	
WOOD	BARK			WOOD	BARK	WOOD	BARK	WOOD	BARK	WOOD	BARK
7	8	Sept. 1, 1925	Check	55.1	16.6	50.2	44.6	224.6	49.1	204.7	132.2
58	59	Apr. 4, 1926		58.2	15.7	59.7	55.0	212.0	48.8	217.7	170.9
89A	89B	June 30, "		37.5	14.9	61.2	56.6	172.3	44.6	280.9	169.8
98	99	Aug. 15, "		46.3	17.2	54.4	56.1	201.6	48.4	237.2	157.8
185	186	Nov. 1, "		57.9	15.5	63.6	57.4	214.8	45.4	235.9	168.7
9	10	Sept. 1, 1925	NPK	29.7	7.5	29.7	23.6	134.2	31.4	134.2	98.3
50	51	Apr. 4, 1926		29.6	3.4	34.9	13.6	119.5	25.3	140.8	100.0
90A	90B	June 30, "		11.4	4.4	16.8	23.1	93.6	20.9	137.2	108.9
94	95	Aug. 15, "		18.0	6.6	18.7	21.5	121.3	25.6	126.4	83.6
182	183	Nov. 1, "		26.0	3.6	26.4	11.7	108.7	23.8	110.5	78.1
1	2	Sept. 1, 1925	NP	41.5	10.9	34.3	30.3	162.2	41.7	134.4	102.1
82	83	Apr. 4, 1926		37.4	4.6	40.0	15.4	136.2	32.5	145.5	109.2
85A	85B	June 30, "		15.3	6.5	20.6	23.6	119.1	31.3	160.0	112.9
110	111	Aug. 15, "		22.0	8.3	23.4	21.5	122.6	32.5	130.4	84.6
194	195	Nov. 1, "		30.0	5.8	28.5	14.4	110.0	31.7	104.5	79.1
3	4	Sept. 1, 1925	NK	41.6	9.8	44.8	33.1	159.6	34.2	171.6	117.5
74	75	Apr. 4, 1926		35.6	5.4	42.6	24.5	143.7	30.1	172.0	136.3
86A	86B	June 30, "		16.3	5.3	31.2	30.4	99.3	24.4	190.5	139.1
106	107	Aug. 15, "		26.0	7.0	31.6	27.2	125.2	29.6	151.8	114.3
191	192	Nov. 1, "		24.1	5.1	34.5	20.6	111.0	27.4	158.6	110.5
5	6	Sept. 1, 1925	PK	37.4	12.6	36.3	31.8	164.8	44.8	159.9	113.1
66	67	Apr. 4, 1926		37.6	9.7	41.4	33.7	142.2	38.1	156.9	132.2
88A	88B	June 30, "		30.2	8.9	55.6	35.9	123.1	33.7	226.6	135.6
102	103	Aug. 15, "		45.2	10.9	46.4	36.1	194.8	38.8	200.1	128.3
188	189	Nov. 1, "		38.0	11.1	47.1	35.9	138.3	37.0	171.2	120.2
11	12	Sept. 1, 1925	N		13.2	33.3	32.9	......	46.3	158.6	115.5
42	43	Apr. 4, 1926		44.5	9.8	40.8	30.1	148.3	39.7	161.5	121.9
91A	91B	June 30, "		...	7.7	34.0	29.2	.....	32.7	207.4	127.6
95	96	Aug. 15, "		26.9	10.8	31.0	35.0	141.3	40.9	162.5	133.0
179	180	Nov. 1, "		33.3	10.9	42.3	34.3	136.1	39.7	166.8	125.1
13	14	Sept. 1, 1925	P	.....	16.6	45.4	43.5	.....	49.4	188.9	129.3
34	35	Apr. 4, 1926		55.2	15.4	48.6	55.5	208.9	46.5	184.0	167.5
92A	92B	June 30, "		....	12.7	50.1	48.6	.....	41.3	255.5	158.2
97	98	Aug. 15, "		44.4	15.9	42.1	50.6	218.3	46.3	206.8	147.5
176	177	Nov. 1, "		63.4	14.7	51.4	55.4	224.9	42.0	182.2	158.4

IV. Discussion of results

1. GLUCOSE AND SUCROSE

Throughout the growth cycle the concentration of glucose is very much greater in the bark than in the wood; this holds for all treatments. Both glucose and sucrose decrease rapidly during the early period of rapid growth and accumulate again as growth acceleration diminishes. But the concentration of glucose (especially in the bark) in the tissues examined of the "no-nitrogen" trees is reduced considerably below those trees which were treated with sodium nitrate.

The ratio of sucrose to glucose indicates that inhibition of invertase activity is quite marked in the trees which have not received applications of nitrogen. Table X gives these data for the bark during the period of active growth.

TABLE X

SUCROSE-GLUCOSE RATIO

TREATMENT	APRIL 4	JUNE 30	AUGUST 15
Check	1.00	1.90	1.54
NPK	0.50	0.18	0.28
NP	0.55	0.28	0.39
NK	0.52	0.30	0.55
PK	0.70	0.60	0.92
P	0.93	2.50	1.10

The addition of nitrogen has resulted in marked effect on the activity of amylase, with concomitant increase in starch utilization. The data for the bark are brought together in table XI.

TABLE XI

STARCH-GLUCOSE RATIO

TREATMENT	APRIL 4	JUNE 30	AUGUST 15
Check	12.50	29.03	23.45
NPK	1.84	3.60	3.30
NP	2.08	3.71	3.41
NK	2.63	4.28	4.36
PK	8.26	8.88	14.12
N	4.26	4.58	4.61
P	14.80	25.50	17.03

These results lend added interest to SPOEHR's (**178**) view that the supply of simple sugars above that required for the normal respiratory activity is one of the factors necessary for growth, and also to LOEW's (**99**) attempt to associate blossom bud formation with a definite concentration of sugars.

2. TOTAL SUGARS

In all treatments the minimum concentration of sugars in the tissues examined is reached during the first period of active growth. Inasmuch as a minimum at this period has also been observed in other experiments (**69**, **122**, **211**), in which the external environmental factors are very different from those of the present experiment, it would appear that this phenomenon is independent of external factors. The concentration of total sugars in trees which did not receive added nitrogen is much lower than in the nitrogen-treated trees. The concentration of total sugars in the tissues examined as related to treatments is in the following descending order: NPK > NP > NK > N > P > check.

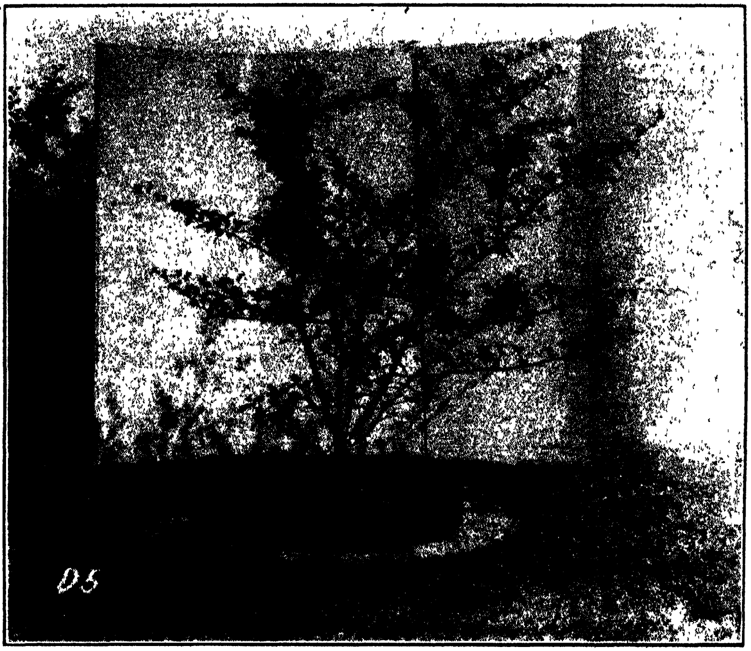

FIG. 4. Typical tree growing in sod treated with the two nutrient salts: K_2SO_4 and $CaH_4(PO_4)_2 \cdot 2H_2O$.

3. STARCH

The variations in the distribution of starch throughout the cycle follow the same general course observed in other investigations with fruit trees

(**69, 122**). Comparisons in this respect with other investigations have to be made with caution, however, because only a few investigators using macroscopical methods have separated bark from wood. SWARBRICK (**194**) concludes from a review of previous work that the seasonal change in starch content shows on the whole two maxima and two minima, the maxima occurring, respectively, at leaf fall and very early in the spring; the minima at the time of maximum leaf growth and early winter. Examination of the results (table VI) of the present experiments shows a distinct lag of the starch changes in the wood compared with those in the bark, so that the dominant phases of the starch cycle in the wood are not the same as those for the bark. This fact has to be considered in any attempt to relate the starch cycle to other physiological phenomena, *e.g.*, to cambium activity and xylem differentiation.

FIG. 5. Typical tree growing in sod treated with the two nutrient salts: $NaNO_3$ and $CaH_4(PO_4)_2 \cdot 2H_2O$.

The great decrease in starch which occurs in the bark—but of the nitrogen-treated trees only—is associated with swelling of the buds. This rapid utilization of starch in the spring is characteristic of the nitrogen-treated trees. Moreover, the accumulation of starch in the bark of all trees at the period of cessation of active growth and the accumulation in the wood later on in the season suggest that starch accumulation may be the cause of retardation of growth.

The starch content of the trees which received nitrogen is at a much lower level throughout the cycle than that of the trees which received no sodium nitrate applications. The trees which did not receive nitrogen show relatively small fluctuations in starch content throughout the cycle.

In this experiment there has not been observed any great deposition of starch in the cortical region of the bark found by some investigators (**161**). Microscopical examination at various times showed that the starch was distributed between the parenchymatous cells of the cortex, phloem, and medullary rays. The pith was packed with starch grains, although it was usually the last part to store it. A similar behavior has been noted by GARDNER (**44**) in the Bartlett pear. The relation of the concentration of starch to the treatments given is in the following ascending order: NPK < NP < NK < N < PK < P < check.

Energy relations of glucose and starch

Glucose and starch may be regarded as two closely related forms of potential energy (**229**). Starch is physically labile, chemically stable, non-diffusible, and non-dialyzable. Glucose, on the other hand, is chemically labile, physically stable, but diffusible and dialyzable. In the relatively low glucose concentration of the cell plasma an equally low intensity of potential energy exists, whereas starch contributes a highly concentrated form of glucose with a corresponding high intensity of potential energy. If one function of the protoplast is to act as a condensation catalyst with power of selectively removing sugars from dilute solution, this may indicate the reason why the determination of starch may be of such diagnostic value. If this view be correct, then the accumulation of starch would be the direct *cause* of growth retardation and not, as HARTWELL (**59**) suggests, the *result* of inhibited growth.

4. HEMICELLULOSES

The physiological function of this important class of structural cell wall substances is still obscure, but there is evidence to show that in many plants this group may function as a reserve material (**93, 129, 135**). Thus LEUKEL (**93**) found wide fluctuations in the amount of hemicelluloses in pasture and forage plants, and NIGHTINGALE (**135**) found a considerable decrease in hemicelluloses in tomato plants during active growth. It must be borne in mind, however, that the sugars and starches are the more labile and, therefore, are the more indicative of immediate seasonal changes.

GERHARDT (**45**), NORMAN (**140, 141, 142**), O'DWYER (**144, 145, 146**), and SCHRYVER and his co-workers (**28**) have, in recent years, added much to our knowledge of the composition of these reserve substances. The results of these workers indicate that the hexosans (galactans, glucosans, and mannans) and pentosans (arabinans and xylans) contained in the cell walls constitute the greatest fractions in certain of the hemicelluloses. The former especially predominate in kernels of seeds poor in starch and fat. There is evidence (**45, 144, 145, 146**) that many of the hemicelluloses contain acid groups of the "uronic" and "glucuronic" acid type.

The hemicellulose of one-year old apple wood examined by GERHARDT (**45**) contained 15 per cent. lignin, 70 per cent. pentose sugars, 5 per cent. ash, and 5 per cent. moisture. No hexoses were identified. The pentosans (d-xylose and l-arabinose) were present in the molar ratio 7:1. However. TOTTINGHAM, ROBERTS, and LEPKOVSKY (**210**) reported that the wood of bearing spurs of apple trees contained xylose, glucose, and galactose. The preliminary hydrolysis of four products isolated from wheat bran by NORRIS and PREECE (**143**) consist of (1) a glucosan, and (2) substances containing pentose residues.

5. DO PRESENT METHODS OF HYDROLYSIS ADEQUATELY REPRESENT RESERVE MATERIAL?

The question has arisen as to whether our present methods of hydrolysis with weak or strong acids hydrolyze the reserve hemicelluloses of apple trees. BRADBURY and ROBERTS (**13**) conclude that they do not. The methods at present in use are based on the work of LECLERC DU SABLON (**92**) and of SCHELLENBERG (**169**). In apple tissues, BRADBURY and ROBERTS (**13**) found that these acids act only upon the young xylem, phloem, and cambium cells and the primary walls of other cells rather than upon the reserves occurring as cell wall thickenings. This may account for the results given in table VI, which show that the seasonal fluctuations in the hemicelluloses are relatively small and, therefore, do not afford a very sensitive index of carbohydrate changes throughout the cycle. This whole problem needs clarification.

In the present experiments hemicelluloses accumulate in the fall but no regularity or trend is observed when all the treatments are considered. The sequence of changes in the hemicellulose fraction parallels to some extent the fluctuations in starch content, but the changes in the concentrations of the latter afford, as already described, a much more sensitive index of the nutritive conditions. The reciprocal relation noted by MURNEEK (**129**) in the reproductive organs of the apple between hemicelluloses and total sugars and also starch is not very definitely defined in the tissues examined in the present investigation. Hemicelluloses are much lower in the bark than in the wood, and are generally highest in the bark of the trees which did not receive nitrogen.

6. TOTAL AVAILABLE CARBOHYDRATES

Accumulation of total available carbohydrates occurs in all trees when growth begins to slacken. The relation of the concentration of total available carbohydrates to the treatments given is in the following ascending order: NPK < NP = NK < N < PK < P < check.

The leaves were yellowish green in the trees without nitrogen additions, a characteristic of high carbohydrate and low nitrogen conditions.

7. TOTAL NITROGEN

The distribution of the nitrogen throughout the cycle is very similar to that found in the earlier experiments of the writer (**201, 202**), the characteristics of which are a high concentration of nitrogen in the spring, a decrease during the period of active growth, and accumulation in the fall. The concentration of nitrogen in the trees to which sodium nitrate was applied is much higher than in the trees which did not receive any added nitrogen. Thus, in these "no-nitrogen" trees the range is from 0.151 to 0.532 per cent. in the wood and from 0.72 to 0.98 per cent. in the bark, compared with 0.44 to 0.815 per cent. and 0.89 to 0.48 per cent. in wood and bark, respectively, of the trees which received sodium nitrate. The concentrations of nitrogen in the tissues examined from the different treatments are in the following descending series: NPK > NP > NK > N > P > check.

It has already been pointed out (p. 415) that the application of sodium nitrate has resulted in a marked increase in the utilization of starch and also (but in a smaller proportion) of the "hemicellulose" fraction. The omission of nitrogen from the complete fertilizer combination (NPK) has resulted in a *decreased* and not an *increased* absorption of the remaining elements. The significance of this has been discussed by the writer in other papers (**206, 208**).

8. CARBOHYDRATE-NITROGEN RELATION

The relationship of this ratio to the vegetative and reproductive responses of plants has dominated the field of horticultural research during the past decade (**6, 62, 63, 69, 71, 82, 83, 84, 135, 136, 155, 157, 167, 232**). A summary of the American investigations on this subject up to 1924 has been given by HOOKER (**71**). The claims to the existence of a positive correlation between this ratio and the vegetative and reproductive responses of plants, however, have not been allowed to pass unchallenged (**6, 232**). The whole problem is essentially related to the question of physiological balance.

A. PHYSIOLOGICAL BALANCE.—The concept of physiological balance in metabolic processes has played a considerable rôle in modern investigations in physiology (**206**). Viewing the problem with respect to the internal conditions of the plant, one may, with PEIRCE (**150**), take the position that from the observed operation of natural laws a shift in the equilibrium conditions within the living cell might be brought about *by any external factor* that influences the rate of any one of the multitude of chemical reactions taking place. Such disturbance in normal metabolism might be either temporary or permanent. We shall return to PEIRCE'S view relative to the external influence affecting fruiting later.

B. NON-EXISTENCE OF SPECIFIC CARBOHYDRATE NITROGEN VALUES FOR A GIVEN RESPONSE.—Although KLEBS (**79, 80**) made many valuable observations on the effect of alteration of external factors on blossom-bud formation, it would appear that FISCHER (**42**) was the first to suggest the possibility of using the carbohydrate/nitrogen ratio as a means of diagnosis, although no quantitative data were reported by him. His observational data led him to conclude that flowering was associated with a very high carbohydrate/nitrogen ratio whereas a low carbohydrate/nitrogen ratio favored vegetative growth.

Although there is no suggestion in the work of KRAUS and KRAYBILL (**82**) of any *specific ratio* of carbohydrate/nitrogen for any response, attempts have been made to establish limiting values and even exact mathematical ratios (**54**) between the available carbohydrate and available (total) nitrogen for a particular response. Frequent citations of GURJAR'S paper appear in the literature, but it has unfortunately been published only in abstract form. On the other hand, there is substantial experimental evidence (**62, 154**) to refute the association of a *specific* carbohydrate/nitrogen ratio with the flowering or the vegetative condition, even in the case of plants of the same species growing under different environmental conditions. Thus, a comparison of the carbohydrate/nitrogen ratios in the case of apple trees in New Hampshire (**154**) and Oregon (**62**) shows that the non-bearing spurs of the former and the most fruitful spurs of the latter investigations had the same carbohydrate/nitrogen ratio. Moreover, bearing spurs from the "sod" plot, which produced no fruit buds, in KRAYBILL'S and POTTER'S experiments (**84**) had the same carbohydrate/nitrogen ratio as the non-bearing spurs on the "nitrate" fertilized plot.

C. FOOD SUPPLY AND BLOSSOM-BUD FORMATION.—In the present investigation fruit-bud formation is definitely associated with such external factors as the supply (concentration) of nitrogen, phosphorus, and potassium. This result is in accordance with empirical deductions of the earlier practical commercial growers and with the later more scientific field observations of GOURLEY (**50**) and of DRINKARD (**36**), that the supply of nutritive elements from the soil is *under most field conditions* the factor of greatest importance in fruitfulness. This fact is also shown by the results of the numerous experiments with laboratory control similar to those initiated by KRAUS and KRAYBILL.

The fact, however, that stunted plants of a grain field and fruit trees about to die from mechanical injury and disease may, in a season of insufficient rainfall, put forth bloom out of all proportion to the food supply, leads PEIRCE (**150**) to the view that increased absorption of mineral elements from the soil and consequently the potential supply to the *bud primordia* is not always a concomitant of fruiting. The experiments of KLEBS (**79**) leave no doubt that fruiting under the conditions of his experiments in vigorously vegetative plants was brought about by varying the supply of nutrients with changes in the illumination. In one of his experiments, for example, this was effected by lowering the concentration of salts and by increasing the illumination. But PEIRCE (**150**) would distinguish between light sufficient for photosynthesis and light as a stimulus to fruiting: "while reproduction may be accomplished without exposure to more light than sufficient for photosynthesis, the more vegetative modes will be the ones developed, the formation of the sexual organs taking place only after sufficient stimulation by light" (**150**). It is possible, nevertheless, that in the experiments of KLEBS and in the cases cited by PEIRCE, fruiting may have resulted when the proportions between available carbohydrates and nitrogen reached definite limiting values. More light would be thrown upon the interpretation of such phenomena if analytical data were available with which plants in the conditions just described could be compared with the results of experiments in which correlations of response with internal conditions are made.

D. EXTENSION OF CONCEPTION OF BALANCE TO OTHER NUTRIENT ELEMENTS. —The importance of the nitrogen supply in the maintenance of a proper relationship between the amount of vegetative and reproductive activities which has long been observed in agricultural practice, especially in fruit growing, can be accounted for by the fact that nitrogen is usually the primary limiting factor under field conditions (**71, 167**). From this viewpoint, the conception of balance may not be confined to the relations between carbohydrates and nitrogen only, but may be extended to balances between other elements or complexes, whichever may be the limiting factors to growth under the particular conditions of growth (**167, 205, 206**).

In the present investigation, and also in those reported by other investigators (**69**), carbohydrate accumulation has resulted from a deficiency of nitrogen; but accumulation of carbohydrates can result also from a deficiency of other mineral elements, especially potassium or phosphorus (**37**). That both potassium and phosphorus have exercised influence on the synthesis of carbohydrates in the present investigation is not open to question. The carbohydrate/K_2O and the carbohydrate/P_2O_5 ratios in the present investigation are shown in table IX. In relation to treatment the former follows the descending series: check $=$ P $>$ PK $=$ N $>$ NP $>$ NK $>$ NPK, and in the latter the order is: check $>$ P $>$ PK $=$ N $>$ NK $>$ NP $>$ NPK. The significance of this order of the ratios will be discussed later.

E. EXPERIMENTS OF ARTHUR, GUTHRIE, AND NEWELL.—These investigators, in an extensive investigation (**6**) on the carbohydrate/nitrogen ratio as related to flowering and length of day, concluded that flowering, under the conditions of their experiments, could be initiated by a long or short day, depending on the species or variety, and that the carbohydrate/nitrogen ratio for a given species varied within wide limits *independently of flowering.* From the results obtained by them, for example, on *Lycopersicum esculentum,* these investigators concluded that the "ratio of carbohydrate to nitrogen has little or nothing to do with fruiting."

In relation to these experiments by ARTHUR and his colleagues there are two important points to be considered, either of which would necessarily invalidate their conclusions just mentioned:

(1) *That in order to establish the utilization of an element or compound apart from that of mere absorption, the determination of the total amounts of materials as determined by chemical methods at any one period does not differentiate between the amount available for synthesis and the amount that may have been utilized. This distinction may be determined with any degree of certainty only by means of time-absorption curves* (**208**).

(2) *That conclusions with respect to the "regulation" of the absorption of an element can be made only when the effect of varying the concentration and ratios of the other principal (dominant) nutrient elements on the absorption of the element in question is known* (**206, 208**). *In view of these facts it may be questioned whether the conclusion of these investigators that "a plant such as corn is so able to regulate the amount of nitrate taken in that it is difficult to induce this plant to take up enough nitrate to increase the total percentage of nitrogen in the plant" can be maintained.*

ARTHUR, GUTHRIE, and NEWELL finally suggest "that a study of enzymes or other substances present in the very small amounts in the growing tips or elsewhere offers much more promise than gross carbohydrate and nitrogen fractions in various plant organs, in explaining the mechanism of light in initiating flowering and fruit production in the plant." Studies of this nature, especially of the type conducted by WENT (**223**), are no doubt highly desirable. It is difficult to understand, however, in what way "the study of enzymes or other substances present in very small amounts in the growing tip" will assist in determining the *causal factor* of flower bud formation. There is little doubt that much of the present confusion with regard to the relationship of carbohydrates and nitrogen to flowering and fruiting is the result of a failure, in many cases, to recognize that the ratio itself is not a *causal factor* but only the resultant of numerous antecedent unknown factors.

It is difficult to escape the conclusion from the results of the present investigation that the carbohydrate/nitrogen ratio, in particular the starch/nitrogen ratio, under ordinary field conditions serves as one of the most sensitive measurable indices of physiological balance that are at present available. In the present experiment the carbohydrate/nitrogen ratio in ascending order is $NPK < NP < NK < N < P <$ check.

F. APPLICATION OF THE LAW OF MASS ACTION.—The concept of the reversibility of the metabolic processes of the nitrogen complexes as a function of the active mass of carbohydrates accords with the known facts of the protein-sparing action of carbohydrates (**180**). And the work of PRIANISHNIKOW (**158, 159, 160**), CHIBNALL (**23, 24, 25, 26, 27**), and also of the writer (**201, 202**) suggests the possible mechanism of this reciprocal relationship between carbohydrates and nitrogen. The final and intermediate stages of the N equilibrium may be represented: "rest" N $\rightleftarrows$ proteins $\rightleftarrows$ amino acids $\rightleftarrows$ amides $\rightleftarrows$ ammonia. The direction of the reactions would be to the right when the active mass of carbohydrates is low and to the left when the carbohydrate concentration is high (**159**).

9. ASH

The ash results present no special problem for discussion. The results consistently reflect the treatments given and are in accordance with LIEBIG's Law of the Minimum. Thus, the series in descending order of magnitude is $NPK > NP > NK > PK > N > P >$ check.

10. POTASSIUM

A. MECHANISM OF THE ACTION OF POTASSIUM.—Recent investigations (**75**) point to the fact that the mechanism of the action of potassium may be very complex—the resultant of photoelectric as well as of radioactive properties. In this connection it is of interest to note that LORING and DRUCE (**101**) found that the potassium chloride obtained from potatoes fertilized with KCl had an atomic weight of 40.5 ± 0.1. A qualitative test showed this sample to be more radioactive than ordinary KCl, indicating a possible biological enrichment of K^{41} in the potato plant. This difference in the physiological effect of an isotope may be related to difference in the degree of hydration of the ions (**208**).

B. Rôle of potassium.—A review of investigations on the functions of potassium will be presented in detail by the writer in another paper. In spite of a prodigious amount of work the mechanism of the action of this element is not yet known. Why does it differ so remarkedly in its action from the chemically related element sodium? Certain physiological effects of potassium, such as its stimulating (catalytic) action on the photosynthetic mechanism, are now well established (**14, 48, 53, 60, 110, 115, 126, 137, 138, 170, 187, 192, 222, 227, 228**). Indeed, Stoklasa and his school (**187, 188, 189, 190, 191**), who have carried out the most extensive and critical experiments on the subject, maintain that synthesis of carbohydrates is possible only in the presence of the potassium ion. The presence of higher concentrations of potassium in meristematic tissues than in other tissues is regarded by some investigators as evidence of a direct or indirect function in mitosis (**104, 137**).

C. Effects of potassium deficiency.—If the results obtained with excised leaves are comparable with the living plant as a whole, as the evidence obtained by Gregory and Richards (**53**) would indicate, it would appear that the effects on assimilation of a deficiency of each of the principal nutrient elements is specific for each element. Table XII summarizes the results obtained by Gregory and Richards.

TABLE XII

RELATIVE RESPIRATION AND ASSIMILATION INDICES OF BARLEY IN COMPLETE AND DEFICIENT NUTRIENT SOLUTIONS

Treatment	Respiration	Assimilation	
		Low light intensity	High light intensity
Complete nutrient solution	Normal	Normal	Normal
Nitrogen deficient	Subnormal	Normal	Subnormal
Phosphorus deficient	Normal	Slightly supernormal	Slightly supernormal
Potash deficient	Supernormal	Subnormal	Subnormal

Thus, only the potassium-deficient leaves showed an initial fall in the assimilation rate equally marked at low as well as high light intensity. The rate of diffusion of CO_2 into the cell up to the chloroplast surface, therefore, would seem to depend upon the potassium concentration, possibly through its rôle in determining the bicarbonate content of the leaf.

It is of interest to compare these results with the nutrient culture experiments of MACGILLIVRAY (**106**) and of JANSSEN and BARTHOLOMEW (**77**). The former found that a deficiency of phosphorus and the latter that a deficiency of potassium resulted in increased percentage of dry matter. From this JANSSEN and BARTHOLOMEW concluded that "up to a certain concentration the potassium ion aids carbon assimilation. If, on the other hand, the concentration of potassium is increased carbon assimilation is again decreased." These anomalous results emphasize a point that plant physiologists cannot ignore; this is that experiments planned to determine the rôle of any of the nutritive elements are of little value unless attention is paid to the question of physiological balance (**208**).

If, moreover, the defense mechanism of the plant against an excess absorption of an ion such as potassium is that of precipitation, as suggested by some investigators (**104, 214**), such regulatory ability must be very limited. The work of SEKERA (**174**) is important in this connection. The clear-cut evidence from SEKERA'S carefully planned experiments on barley is that normal plants are able to regulate the amount of an ion absorbed in excess of that utilized in metabolism by expulsion of that element into the soil *via* the roots. This has been discussed in detail by the writer in another paper (**208**).

D. POTASSIUM AND TRANSLOCATION OF STARCH.—Although there is considerable evidence to indicate that potassium is effective in increasing the translocation of starch (**75, 76, 77, 110, 138**), experiments may also be cited (**134, 176**) the results of which have been interpreted as indicating that translocation of starch is independent of potassium salts. Thus, NEWTON (**134**) found in water cultures that almost twice the weight of potato tubers was obtained in minus-K solutions compared with those from the complete culture solutions. The weight of tops and roots, however, was slightly in favor of the latter cultures. However, in the minus-Ca series, in spite of leaving a 25-gm. seed piece attached to the seedling, the plants died. Potato tubers have relatively a very high content of potassium and a very low content of calcium. Hence, as NEWTON points out, his experimental evidence leads to the conclusion that the beneficial results of omitting potassium were due to the increased absorption of calcium. The reciprocal action of potassium and calcium has been observed in a number of experiments (**208**). These anomalous results accordingly may be attributed to a lack of physiological balance in the potassium cultures. The results of SMITH and BUTLER (**176**) may be similarly interpreted. The experiments of the latter do not indicate that translocation of starch is independent of potassium but that the potassium present in the seed is relatively efficiently utilized during the very short duration of these experiments (21 days).

E. Effect of potassium additions in the present experiments.—

(1) *On the leaf color.*—None of the typical symptoms of potassium deficiency, *viz.*, yellowish green leaves (the color is usually dark green if sodium is also deficient) with purple veins or browning of the periphery, were observed in any of the trees.

(2) *On the enzyme system.*—Many experiments (**33, 40, 41, 175, 184, 193**) indicate that potassium has a marked effect on the enzyme systems of the plant and that the effects are specific for different enzymes. In the present experiments potassium additions have increased the activity of amylase and of invertase (table VI). The marked effect of potassium in increasing the utilization of starch in these experiments is also apparent from the data given. Thus, the starch/K_2O ratios are in the following ascending order with respect to treatments: NPK < NP < NK < N < PK < P < check. It is of interest to note that Reed and Haas (**163**) observed that orange trees can function with less potassium than they ordinarily absorb. Trees receiving no added potassium were able to grow for 17 months subsisting presumably on the potassium in the trees at the time they were planted.

(3) *On the absorption of nitrogen and phosphorus.*—Potassium additions have increased the absorption of both nitrogen and phosphorus in the tissues examined (**205, 206**) and, as a later paper will show, this statement may be generalized to the absorption of these elements by the entire tree.

(4) *On the potassium/nitrogen ratio.*—Wallace (**219, 220**) has emphasized the significance of this ratio. In his sand cultures, leaf scorch (tip burn) of trees growing with insufficient potassium increased with each increment of nitrogen in the nutrient solution; at relatively high K/N ratios leaf scorch (tip burn) did not develop. Bronzing of the leaves of citrus trees growing in nutrient solutions having a low K/N ratio was also reported by Reed and Haas (**163**).

Certain investigators (**39, 77**) have noted a reciprocal relationship between the content of the plant with respect to nitrogen and potassium. The whole question of the mechanism of the reciprocal relationship between nitrogen, phosphorus, and potassium in relation to their absorption by plants has been discussed in detail by the writer elsewhere (**206, 208**). It may be pointed out here, however, that no generalizations are possible with respect to the K/N ratio because the absorption of these elements by the plant is a function of the level of nutrition, *i.e.*, rate of supply of nutrients (**206, 208**). Accordingly, results may show either a positive (as in the present experiment) or a negative correlation (**39, 77**) with respect to the amount of these elements absorbed by a plant.

11. PHOSPHORUS

A. RÔLE OF PHOSPHORUS IN RESPIRATION.—The experiments of both LYON (**102**) and SPOEHR (**179, 181**) *in vitro* indicate that phosphorus may act as an accessory accelerator of respiration. The starch/P_2O_5 and the carbohydrate/P_2O_5 ratios (table IX) of the trees of the various treatments in the present field experiments also suggest such an effect. The isolation of a reducing hexosemonophosphate by NEUBERG (**131, 132**) from bottom yeast and by BARRENSCHEEN and PANY from *Elodea canadensis* (**12**) affords very positive evidence that phosphates play a rôle in the intermediate stages of the carbohydrate metabolism of plants.

B. ABSORPTION OF PHOSPHORUS.—In the present experiments the absorption of phosphorus by trees which did not receive phosphorus application and by those trees which received phosphorus additions unaccompanied by the other principal nutrient elements is relatively low. A significant increase in the absorption of added phosphorus has occurred only by the addition of potassium or nitrogen or both, *i.e.*, in the NP and NPK treatments. Since a rapid "fixation" of $PO_4^{\equiv}$ ions takes place in this Hagerstown clay loam soil (**197**), this increase in the absorption of phosphorus may be interpreted as the result of an increase in the solubility of phosphorus following a decrease in the already existing potential difference between the micellar and intermicellar soil solution by the K^+ and NO_3^- ions, in accordance with the GIBBS-DONNAN law (**1, 49, 116, 183, 185, 208**).

C. STARCH/P_2O_5 RATIO.—Both the starch/P_2O_5 and the available carbohydrate/P_2O_5 ratios indicate that phosphorus may act as an accessory accelerator of respiration only if adequate supplies of nitrogen and potassium are present. Hence, it follows that in soils, such as the Hagerstown clay loam used in this experiment, which are relatively low in nitrogen and phosphorus albeit high in available potassium, the application of a carrier of phosphorus (P) or of phosphorus and potassium (PK) would be ineffective to permit sufficient utilization of carbohydrates for normal growth and reproduction unless nitrogen is also applied.

D. N/P_2O_5 RATIO.—In the leaves of *Vitis vinifera*, LAGATU and MAUME (**87**) found a linear relationship between the nitrogen and phosphorus contents. This would be expected if all the phosphorus is in organic combination as nucleic acid, lecithin, and allied substances. The nitrogen and phosphorus would then be present in the same molecule. This linear relationship does not exist in the tissues examined in the present experiment, for wide seasonal changes in the N/P_2O_5 ratio occur in all treatments. This indicates that the large and variable proportions of the phosphorus are present as nitrogen-free compounds.

12. WATER RELATIONS: PHYSIOLOGICAL DRYNESS OF TISSUES

The position of the water balance within a plant is determined by the power of the roots to maintain the supply and the power of the leaves to resist excessive loss. The rate of water loss, if the plants do not differ internally in some manner affecting transpiration, will depend upon the nature of the leaves and the area of foliage produced. Many experiments (**22, 58, 98, 173**) indicate that the nearer to the optimum the physiological balance of the nutrient solution the smaller becomes the transpiration per unit of dry matter produced.

A. POTASSIUM AND PHYSIOLOGICAL DRYNESS OF TISSUES.—From the observation that potassium-starved fruit trees contained less water than plants receiving potassium additions, WALLACE (**221**) postulates that physiological dryness of tissues may be a characteristic of plants deficient in potassium. JAMES (**76**) also found a significant correlation ($P < 0.01$) between potassium and water content throughout the growth period of *Solanum tuberosum* in the presence of chloride ions but not in their absence, and GREGORY and RICHARDS (**53**) found even a decreased water content associated with additional potassium at moderate concentrations. These facts would seem to indicate that other causes than osmotic pressure and solution tension are at work. Nevertheless, the influence of such a strongly hydrated element as potassium upon the swelling properties of gels must be considerable. These results are of interest with respect to the relation of potassium to chlorophyll formation and carbohydrate synthesis discussed earlier in this paper. In this connection, it is interesting to note that the work of DASTUR and BUHARIWALLA (**32**) shows a positive correlation between the chlorophyll content and water content. Both rise as the leaf grows to maturity and both fall (the water content most) as the mature leaf grows old.

In the present experiments, nitrogen appears to be the greatest factor in decreasing physiological dryness of tissues (table V). This may be due to the influence of sodium nitrate additions on the increased uptake of potassium. The leaves and one-year and season's branch growth of trees receiving additions of all three nutrients (NPK) have the highest water content. The series in descending order is: $NPK > NP > NK = N > P >$ check.

B. BAKHUYZEN'S PHYSICO-CHEMICAL INTERPRETATION. — BAKHUYZEN (**10**) has sought to interpret the facts of permeability, of ecological behavior, of differentiation and adaptation, and also of the life cycle of plants from the colloid chemical view-point. The water content, therefore, plays an intrinsic rôle in this theory. If the data of MOMMSEN (**124**) and NAYLOR (**130**) can be generalized, "external" protoplasm has an isoelectric point at a pH of 4.2–4.5, whereas that of the nucleus is much higher, *viz.*, pH 5.0–5.5. There exists, therefore, a relatively high ζ-potential between "external" protoplasm and the nucleus; in other words, the peripheral plasm is more negatively charged than the perinuclear part. Accordingly, BAKHUYZEN postulates that, as the growth of the cell proceeds, the hydration capacity of the "external" protoplasm increases (increase of negative charge), whereas that of the "internal" protoplasm or perinuclear plasma decreases (decreases of negative charge or increase of positive charge). Since the minimum hydration of a colloid is at its isoelectric point, a coagulation of the internal plasma with consequent loss of hydration water and absorbed solutes occurs as growth proceeds. In this way the vacuole is formed. As the cell grows the difference between the charge (ζ-potential) of the "external" and that of the "internal" protoplasm increases up to the point when the cell begins to age. If the negative charge on the outside of the protoplasm is destroyed, solutions of acids, bases, or salts will nevertheless tend to stabilize the colloid. BAKHUYZEN argues that since OSTERHOUT (**147**) finds that death of protoplasm results in loss of isoelectric charge, there must, in long-living cells, be a force which keeps up this charge during the life of the cell. He postulates that the source of this counteracting force is probably the growth hormones, which increase the hydration capacity of the protoplasm during growth and tend to keep it up after growth in volume is over (**56, 223**). The withdrawal of water and salts from the vegetative parts (**9**) is thus accounted for.

V. Summary

Standard apple stocks vegetatively propagated from a single parent and whip-grafted to scions from a tree of known history were planted in a virgin soil contained in 5 foot by 5.5 foot boiler plate cylinders. These cylinders, 42 in number, were sunk in the ground but were isolated from the surrounding soil by means of a 6-inch layer of crushed limestone. Two years after planting, the trees were subjected to differential fertilizer treatments by the application of different combinations of the pure salts $NaNO_3$, $CaH_4(PO_4)_2 \cdot 2H_2O$, and K_2SO_4, applied in very small (low) concentrations in the spring of each year.

Marked differences in response to treatments were visible within a few months and became progressively intensified as the experiment continued. During the years 1925–1926, analyses of the one year and season's branch growth with respect to certain elements or groups of elements were made at critical periods in the seasonal growth of the trees under sod. The bark and wood were separately examined. The reproductive responses are indicated by the number of blossom clusters formed during this period. *Under cultivation:* NPK, 353; NP, 334; N, 71; PK, 28; NK, 17; P, 13; check, 6.

Under sod: NPK, 328; NP, 145; NK, 102; N, 21; PK, 0; P, 0; check, 0. Branch elongation followed the same order relative to treatment.

The analytical results on the one year and season's branch growth show that:

1. Both "glucose" and sucrose decrease rapidly during the period of active growth and accumulate again when growth acceleration diminishes; this accumulation of simple sugars appears to be a necessary precursor of growth.
2. The concentration of simple sugars in the case of trees which did not receive any sodium nitrate applications is much lower than in the nitrogen-treated trees.
3. Inhibition of invertase activity is marked in the case of the trees which did not receive nitrogen.
4. The starch cycle in the wood lags behind that of the bark. Bud swelling, especially in the nitrogen-treated trees, is associated with a great decrease of nitrogen in the bark.
5. Storage of starch up to the fall in the no-nitrogen trees is relatively very great, but the accumulated starch reserves of the trees which received applications of sodium nitrate rapidly diminish as growth proceeds. In relation to the different treatments, starch follows the ascending order NPK < NK < N < PK < P < check. Storage of starch is intimately connected with cessation of active growth and appears to be the cause of growth retardation in these experiments.
6. The energy relations of glucose and starch are examined and discussed.
7. The literature on the physiological function of the hemicelluloses as reserve material is briefly reviewed. In the present experiments the seasonal fluctuations of this group are relatively small.
8. As in the case of starch, the total available carbohydrates in relation to the different treatments follow the same ascending series: NPK < NP < NK < N < PK < P < check.
9. Total nitrogen decreases rapidly during the period of active growth and accumulates in the fall. The concentration of nitrogen in relation to the treatments follows the descending series: NPK > NP > NK > N > P > check.
10. The addition of nitrogen as sodium nitrate results in a great increase in the utilization of starch. Nitrogen concentration is the principal factor that determined, in these experiments, the utilization of accumulated starch; but increased phosphorus and potassium concentrations in the tissues examined also stimulate additional utilization resulting in increased fruit-bud formation.

11. The literature of the carbohydrate/nitrogen ratio (and also of the starch/nitrogen ratio) in relation to the vegetative and reproductive responses of plants is examined and discussed. It is pointed out that, although no mathematical ratio in the sense of the existence of a specific carbohydrate/nitrogen ratio for a particular response exists for plants growing under different meteorological conditions, nevertheless, as the present experiment indicates, this ratio serves, under the conditions ordinarily met with in the field, as one of the most sensitive measurable indices of physiological balance that are at present available. In the present experiment this ratio shows a definite gradient relative to the treatments in the following ascending series: NPK < NP < NK < PK < P < check.

12. The percentage of ash in relation to treatments is in descending order: NPK > NP > NK > PK > N > P > check.

13. The literature with respect to the rôle of potassium in plant nutrition is briefly discussed. It would appear that the mechanism of the action of potassium is very complex and is probably the resultant of photoelectric as well as radioactive properties.

14. Although the soil used in these experiments has a high potassium content (3.96 per cent. K_2O), the application of K_2SO_4 increased the potassium content of the tissues which has resulted in increased utilization of starch.

15. The reciprocal relationship between potassium and nitrogen of plants found by some investigators is discussed. A positive and not a negative correlation between these elements is found in the present experiment.

16. The application of phosphorus as $CaH_4(PO_4)_2 \cdot 2H_2O$ also resulted in increased utilization of starch. Phosphorus appeared to have assisted in the oxidation of the simple sugars.

17. The amount of phosphorus absorbed by trees which received no applications of a phosphorus carrier, and also by those trees which received phosphorus additions unaccompanied by one of the other nutrient elements, is relatively small. The reasons for this are discussed. Applications of potassium and also of nitrogen have increased the absorption of phosphorus.

18. Both the starch/P_2O_5 and the available carbohydrate/P_2O_5 ratios indicate that phosphorus may act as an accessory accelerator of respiration only if adequate supplies of nitrogen and potassium are present.

19. Although some investigators find that physiological dryness of tissue may be characteristic of potassium-deficient plants, in the present experiment nitrogen has been the greatest factor in decreasing physiological dryness of tissue. Thus, the amount of water in the tissues examined follows the descending series: NPK > NP = N > NK > PK > P > check.

20. The literature on many phases of the interrelationships between the plant and the soil pertaining to the present study is reviewed and examined.

The budding, grafting, and general care of the trees were in charge of Dr. R. D. ANTHONY and Mr. J. H. WARING, of the Department of Horticulture. The growth measurements were made by Mr. LEIF VERNER and Mr. R. H. SUDDS, also of the Department of Horticulture.

THE PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PA.

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RELATION OF AGE AND OF SEASONAL CONDITIONS TO COMPOSITION OF ROOT, PETIOLE, AND LEAF BLADE IN RHUBARB

CHARLES W. CULPEPPER AND JOSEPH S. CALDWELL

(WITH FIVE FIGURES)

Introduction

Members of the genus *Rheum* have been employed in medicine from a period antedating authentic history, and the general distribution of most of the species throughout Europe and North America is due primarily to such use. The employment of rhubarb as an article of food is relatively recent, the earliest reference to such use found by STURTEVANT (HEDRICK **17**) bearing the date 1778; but its use appears to have become rather general shortly after 1800. Excellent descriptions of the several species are given by various writers (**3, 7, 19**) in connection with directions for the cultural treatment of the crop, and there is a rather extensive literature dealing with the chemical composition of the root and the physiological action of the various constituents isolated therefrom which has recently been reviewed in connection with a comprehensive reexamination of the root (**35**). The older literature dealing with the use of the plant in medicine has been exhaustively reviewed by HEUBERGER (**18**). Such chemical studies as have been made of the aerial parts have been concerned with the determination of the food value of the petioles or with the possible toxicity of the leaf blades when employed as food. Apparently no comprehensive physiological or biochemical study of the seasonal development of the entire plant has hitherto been made.

In many respects the rhubarb plant (*Rheum hybridum* was the species studied) lends itself exceptionally well to such investigation. The large size of the several parts and the extremely rapid development of the enormous leaf blades and petioles make it suitable for studies for which large quantities of material and frequent collections are necessary. The size of the leaves facilitates marking, so that collection of material of known age is readily possible. The high acidity and low carbohydrate content of the aerial parts are exceptional, and suggest that the metabolic processes of the plant possess features of interest. In connection with studies upon the food value and cooking and canning qualities of rhubarb, some of which have been reported elsewhere (**14**), it was found that the physiology of the plant presented some unique features. It was therefore considered advisable to undertake biochemical studies for the light which the results might throw upon the problems of its utilization in cooking and in canning, as well as for their general physiological interest. The present paper presents the results of this study.

Review of literature

The literature dealing with the chemistry of rhubarb is concerned, as already indicated, with the food value of the aerial parts or the therapeutic effects of the constituents of the root, and not with the biochemistry of growth and development of the entire plant or with the effect of climatic conditions or plane of nutrition in modifying the character or degree of these changes. The work of STEINMANN (**32**) was devoted to the determination of the titratable acidity of the petiole and leaf blade at various stages of development, to the effect of illumination and darkness upon the acid content, and to the gradient of titratable acidity between leaf mesophyll, veins, and petiole. He reached the conclusion that oxalic acid may be a product of photosynthesis, playing a rôle analogous to that of carbohydrates in ordinary green plants. RUHLAND and WETZEL (**27**) have reported studies upon the plant which they consider indicate that the high acid content is the result of deamination of proteins rather than of partial oxidation of carbohydrates.

There is an enormous literature dealing with the annual cycle of chemical exchanges between leaves and storage organs of woody perennials, and particularly of fruit trees, from which the general character and sequence of these changes can be rather completely determined. This literature has been excellently summarized by GARDNER, BRADFORD, and HOOKER (**16**), and by CHANDLER (**13**). The degree to which the results of such studies apply without modification to the rhubarb plant is questionable, since production of flower stalks and fruits is normally prevented in cultivation, storage of reserves is confined to the underground rhizome and roots, and the aerial parts are remarkable for their consistently high acidity and low content of sugars and acid-hydrolyzable polysaccharides at all stages of their development. In consequence the physiology of the plant would be expected to present some points of contrast with that of woody stemmed perennials and trees on the one hand, and with that of annuals on the other.

There is a considerable literature dealing with the chemistry of development of foliage leaves, chiefly of herbaceous perennials and trees. The results reported by OTTO and KOOPER (**24, 25**), TUCKER and TOLLENS (**34**), SWART (**33**), and LECLERC DU SABLON (**15**) are typical. SWART found that the fall of the leaf in autumn was preceded by a decrease in protein and in nitrogen, phosphorus, and potassium. He reviewed the older literature in considerable detail and presented analyses upon leaves of a great number of trees and perennial shrubs made at two stages, namely, when the leaves were normally green and when autumnal coloration had set in. In all cases the development of coloration is accompanied by very marked decrease in total nitrogen, potassium, and phosphoric acid.

TUCKER and TOLLENS (**34**) collected leaves of *Platanus occidentalis* at intervals of 3 to 4 weeks from June 13 until November 5, and determined water and ash content and the composition of the ash. The water content was highest in the first sample and decreased gradually up to October 8, then rose slightly in the final sample. Ash increased throughout the series. The percentage of silica, calcium, magnesium, and chlorine present in the ash increased steadily through the series; phosphoric acid and potash were highest in the initial sample and fell off gradually up to September 7, then more rapidly to values less than one-half those of the initial sample. Nitrogen showed a similar but more pronounced decrease to one-fourth its original amount. TUCKER and TOLLENS reduced their results to terms of equivalent leaf area, so that the decreases are absolute and not relative. As WEHMER (**38**) had suggested the possibility that constituents of the ash were removed from the older leaves by rains, a portion of the tree was protected by a water-tight cover during rainfall from October 8 onward. The differences between protected and unprotected leaves were without significance. The reduction in potassium, phosphoric acid, and nitrogen was therefore due to transport from the leaves into the wood, not to leaching by rains.

LECLERC DU SABLON (**15**) made determinations of sugars and total carbohydrates, total nitrogen, fat, and water in root, stem, and leaf of chestnut, pear, quince, peach, and willow trees and in raspberries at intervals of 30 to 35 days throughout the year, finding that the total carbohydrate of leaves generally increased somewhat, then fell off very considerably previous to leaf fall. Total nitrogen was maximum in amount in the youngest leaves and decreased rapidly until the leaves were fully grown, then decreased more slowly. Water content was highest in the youngest leaves and decreased with age. Carbohydrates and total nitrogen of root and stem were maximum in October and November, decreased rather rapidly to a minimum in April or May, then increased from June or July to the end of the growing season.

BERTHELOT (**6**) has summarized a considerable number of analyses of various plants at several stages of development, but the distribution of nitrogen among its various forms was not determined.

The work of OTTO and KOOPER (**24, 25**), while primarily carried out with another purpose in mind, shows that there is a progressive decrease in nitrogen content of the leaves of *Syringa vulgaris, Sambucus nigra, Philadelphus coronarius*, and *Aesculus hippocastanum* throughout the season.

The literature in regard to the chemical changes in the leaves of deciduous perennials and trees is in agreement with that here cited in indicating that there is a progressive decrease in total nitrogen content of the leaf from spring to autumn, and that senescence of the leaf is preceded by a complete disappearance of nitrates and a further decrease in nitrogen, phosphorus, and potash as a result of transfer from the leaf to the stem. Concurrently with the decrease in total nitrogen there is a progressive increase in water content of the leaf, which becomes relatively stationary during the period of functional activity and declines as senescence sets in. BENEDICT (**4**), who has summarized the literature on the physiological changes occurring in senescence in leaves, found that old leaves of *Vitis vulpina* had materially lower capacity for absorbing water than young leaves, which he attributed to the increased content of vascular tissue in the old leaves.

Certain annual plants are known to accumulate very considerable quantities of nitrates in their stems and leaves. BOUTIN (**8, 9**) reported in 1873–1874 the finding of large quantities of potassium nitrate in aerial parts of *Amaranthus atropurpureus, A. blitum,* and *A. ruber.* PAMMEL and DOX (**26**) made chemical analyses and microchemical studies of *A. blitoides, A. graecizans,* and *A. retroflexus,* and the results of their total nitrogen determinations ranged between 1.88 and 2.49 per cent. As the quantities of protein indicated by the microchemical tests were in all cases very small, much the larger portion of the total nitrogen was non-protein nitrogen, and a considerable part may have been present as nitrate. These reports prompted further work by WOO (**39**) and its subsequent extension by CAMPBELL (**12**).

WOO made chemical analyses of roots, stems, branches, and leaves of *Amaranthus retroflexus* at three stages of growth, beginning at emergence from the ground and ending just before blooming. CAMPBELL (**12**) continued the work to include the mature stage and also made analyses of 25 other weed species at three stages of development. WOO had found a pronounced accumulation of nitrate nitrogen in the stems and branches with less marked increase in the roots just prior to blooming, which had entirely disappeared from all parts in the mature plant. CAMPBELL found in all the species studied that a somewhat analogous accumulation of nitrate nitrogen occurred in the early stages of growth, reaching its maximum just before blooming, then disappearing in the mature stage. Of the various orders and families represented, representatives of Chenopodiales showed the highest accumulation of nitrate, with Polygonales ranking next (represented by *Rumex crispus, Polygonum pennsylvanicum,* and *P. convolvulus*). When grown in soils very high in available nitrate content, as on piles of stable manure or decayed hog hair, *Amaranthus retroflexus* and

Atriplex patula showed a considerable content of nitrate nitrogen at full maturity. In all the forms used the total nitrogen content of stems, roots, and leaves decreased from the earliest stage to maturity.

Woo found that total carbohydrates, polysaccharides, and non-reducing sugars in roots and stems increased in the first two weeks after emerging from the soil, meanwhile decreasing in the leaves. Just before blooming the relations were reversed, a sharp decrease in these constituents in the root and stem accompanying the increase in the leaves. Total carbohydrate and total nitrogen showed reciprocal fluctuations in each of the three parts, increase in total nitrogen accompanying decrease in total carbohydrate and *vice versa*.

From the results reported by CAMPBELL, it would appear that accumulation of considerable quantities of nitrate in the leaves of annuals, biennials, and perennials whose above-ground portions mature and die before frost is of rather general occurrence in the period prior to blooming; but that the appearance of flowers and seed is accompanied by total or nearly total disappearance of nitrates from leaves and stems.

The work of SCHERTZ (**30**) on mottling of leaves of *Coleus,* taken in conjunction with the earlier work of SAMPSON (**29**), on abscission in the same plant, is of interest in this connection. The normal green leaves of this plant have a somewhat high content of nitrate at all stages of growth up to full size, no material reduction in nitrate content occurring so long as the leaf is functional. Leaves in which mottling had become perceptible were always lower in all forms of nitrogen (and especially in nitrate nitrogen) than normal green leaves; their phosphate content was only one-third that of normal leaves. In leaves in which the abscission layer was in process of formation, SAMPSON's analytical data indicate that there is a reduction in nitrate nitrogen in the leaf blade with a considerable increase in the immediate neighborhood of the abscission layer previous to leaf fall. There was little evidence of any general transport of carbohydrates, phosphates, or organic nitrogen compounds out of the leaf previous to its fall. SAMPSON gives no indication as to the age of his plants nor as to whether they had reached the flowering stage. The photographs accompanying the paper of SCHERTZ (**30**, figs. 5, 6) indicate that the plants from which leaves were taken were producing flowers. It would consequently appear that in *Coleus* no such reduction of nitrate content of the leaves occurs at flowering as was found by CAMPBELL in *Amaranthus* and other weeds.

NIGHTINGALE (**23**) concludes that "nitrates may be stored by the plant within itself until the proper conditions arise for synthesis to other forms of nitrogen." This is unquestionably true. The high nitrate content of young plants and the lower content of the mature plants examined by CAMPBELL may be due to exhaustion of available nitrates within reach of the root system and to utilization of the stored nitrate in the construction of protein, or to the possession of high nitrate-absorbing capacity by the young plant and cessation of such absorption, regardless of supply, as its utilization in growth ceases after production of flowers and seeds.

Summarizing, it would appear that in annuals, nitrates are rather generally accumulated during the period of most active growth and exhausted during development of the reproductive structures.

Experimentation

The purpose of this paper is to record some of the findings in regard to the biochemical changes occurring in the rhubarb plant in the course of its seasonal growth. Particular attention has been directed to the changes in acidity, carbohydrate, and nitrogenous constituents occurring incidental to growth, maturity, and senescence of the aerial parts, and to the effects of the annual march of seasonal conditions upon the character of these changes.

Source of material

The material used in these studies of rhubarb was supplied through the kindness of D. N. Shoemaker and was grown in the variety test plot at Arlington Experimental Farm near Rosslyn, Va. The variety, Ruby, was originated at the Central Experimental Farm, Ottawa, Canada, and was named and distributed for trial by Dominion Horticulturist W. T. Macoun (**20**) in 1923. The planting was made in the spring of 1927 with material obtained by the usual method of dividing the crowns of old plants. The samples were taken in 1928, so that the planting was one year old. Generous applications of well rotted stable manure were made in 1927, but no manure or commercial fertilizer was applied in 1928. The plot was frequently cultivated throughout the season to kill weeds. No flower stalks developed upon any of the plants during the season. The variety is one that does not readily form flower stalks.

Methods of sampling

Four series of samples were taken during the season. The first of these series differed from the others in that it consisted of the first leaves which were developed by the plants in the spring. The plot was inspected on April 21, at which time the leaves were just appearing above ground. Identifying tags were attached to a considerable number of leaves having petioles 2–4 inches in length with the leaf blade not yet completely expanded. These leaves were arbitrarily assumed to be 10 days of age at this time. From the group of leaves so marked, samples were taken at intervals of 10 to 14 days, beginning April 21, up to June 15, at which time the leaves first marked were 65 days of age and were yellowing and dying.

From April 21 onward the plants were inspected at intervals of 5 to 8 days, and a number of developing leaves having petioles 2–4 inches long were tagged at each inspection. This made it possible to collect at one time a series of samples of leaves and petioles of known and varying ages up to senescence. Such collections were made on three dates, June 5, July 6, and October 17, each collection consisting of samples ranging in age from 5 to 8 days up to 60 to 65 days. In consequence, while the first series consisted of petioles and leaves all of which began their development at one time, the later series were made up of material beginning development at various dates throughout the entire period of active growth, from April 11 to October 9.

The samples were in all cases collected about 10 o'clock in the morning, after dew, if present, had evaporated. Each sample was made up of about 40 leaves from 20 plants, collected by pulling the petioles away from the crown, as is customary in harvesting for market. The material was immediately transferred to the laboratory and sampled for analysis as rapidly as possible, being protected from evaporation meanwhile. In taking the samples of leaf blades, the midrib and its lateral branches were removed and discarded, the sample consisting of mesophyll tissue with only the smaller veins, taken from various regions of a great number of leaves. The samples of petioles were prepared by cutting thin sections from upper, middle, and basal regions of each of a sufficient number of petioles to make up the desired quantity. Duplicate 100-gm. samples were taken in all cases. In taking the root samples, an entire plant was taken up, brought into the laboratory, and portions of the root system selected which were as nearly representative of the entire system as a whole as possible. As it was not possible to collect the very fine rootlets, the samples consisted chiefly of the thickened rootstocks and roots in which storage of starch and other substances occurs. The first root sample was taken April 21, at the outset of seasonal activities; the others were taken at the same time as the petiole and leaf samples.

METHODS OF ANALYSIS

The samples were preserved in sufficient 90 per cent. alcohol to make the final concentration 75 per cent. and were heated to boiling to stop enzyme action. The following November the samples were extracted with alcohol, the extracts made up to volume, and aliquots taken for determination of soluble solids, sugars, tannins, and nitrate nitrogen. Acid-hydrolyzable polysaccharides were determined upon a portion of the insoluble residue. Total nitrogen determinations were made upon portions of material dried in an air oven at 70° C. and finished in a vacuum oven.

SUGARS.—Determinations were made before and after hydrolysis with HCl at 70° F. by the MUNSON-WALKER method, the cuprous oxide being determined by the volumetric permanganate method. The results for free reducing sugars are omitted from the tables.

TITRATABLE ACIDITY.—Titrations were made with N/10 NaOH against phenolphthalein and the results expressed as malic acid.

TANNINS.—Titrations were made with N/20 $KMnO_4$ with indigo-carmine as indicator.

ACID-HYDROLYZABLE POLYSACCHARIDES.—The official method of the Association of Official Agricultural Chemists for determination of starch by direct acid hydrolysis was employed. (See footnote, p. 462.)

TOTAL NITROGEN.—Determinations were made by the KJELDAHL method as modified to include nitrate nitrogen.

NITRATES.—The method recommended by the Official Methods for determination of nitrates in meats was followed.

AMINO ACIDS.—Determinations were made by the VAN SLYKE method upon aliquots of the material after preservation in alcohol. In view of the work of WEBSTER (37), it is doubtful whether the results can be considered reliable, but they may have a certain degree of comparative value.

WEATHER CONDITIONS DURING SAMPLING PERIOD

The weather conditions for the 12 months of 1928 are given in table I, which summarizes for each month the rainfall, normal mean temperature, and percentage of the possible sunshine, with departures from the respective monthly normals. The year was somewhat warmer than normal up to April 1, with somewhat deficient rainfall. April had a moderate excess of rain and was below normal in temperature; May was very close to normal in all respects; June and July were deficient in rainfall and drought conditions had become well established by August 11 and 12, at which time rainfall of more than 8 inches occurred, followed by several further rains which brought the total for the month up to 14.41 inches. September was markedly cool and deficient in sunshine; October was dry and warm, as were the following months.

In table II the weather conditions for 40 days preceding the taking of each sample are stated by periods of 10 days each, mean temperature, percentage of possible sunshine, and rainfall having been calculated from the daily records of the Weather Bureau. Certain facts are immediately obvious from inspection of the table.

The first series of samples began development about April 10 and were collected at various times up to June 15. The second series began development at various times between April 21 and June 1 and were taken all at one time on June 5. The period from April 21 to June 15 was one of progressively rising temperature, with sunshine close to the normal and with no marked deficiencies in rainfall. The 10-day period ending April

TABLE I

MONTHLY PRECIPITATION, MEAN TEMPERATURE, AND PERCENTAGE OF POSSIBLE SUNSHINE, WITH NORMALS AND DEPARTURES THEREFROM, FOR THE YEAR 1928; WASHINGTON, D. C.

MONTH	RAIN	NORMAL	DIFFERENCE	TEMPERATURE	NORMAL	DIFFERENCE	SUNSHINE	NORMAL	DIFFERENCE
	inches	*inches*	*inches*	°*F.*	°*F.*	°*F.*	*per cent.*	*per cent.*	*per cent.*
Jan.	2.63	3.38	− 0.75	35.9	33.4	+2.5	65	46	+19
Feb.	2.69	3.42	− 0.73	37.7	35.3	+2.4	58	54	+ 4
Mar.	2.17	3.85	− 1.68	44.1	42.6	+1.5	60	51	+ 9
April	4.49	3.25	+ 1.24	51.9	53.3	−1.4	55	58	− 3
May	4.00	3.83	+ 0.17	63.1	63.7	−0.6	62	61	+ 1
June	2.66	4.13	− 1.47	71.0	72.2	−1.2	53	62	− 9
July	2.17	4.71	− 2.54	78.2	76.7	+1.5	66	64	+ 2
Aug.	14.41	4.01	+10.40	77.0	75.0	+2.0	49	61	−12
Sept.	4.29	3.24	+ 1.05	64.9	68.1	−3.2	43	63	−20
Oct.	0.67	2.84	− 2.17	59.9	57.4	+2.5	61	61	− 0
Nov.	2.00	2.37	− 0.37	49.4	45.2	+4.2	48	56	− 8
Dec.	1.21	3.32	− 2.11	39.2	36.6	+2.6	58	51	+ 7

TABLE II

WEATHER CONDITIONS PRECEDING THE TAKING OF SAMPLES BY 10-DAY PERIODS

SAMPLING	1ST 10 DAYS PRECEDING SAMPLING			2ND 10 DAYS PRECEDING SAMPLING			3RD 10 DAYS PRECEDING SAMPLING			4TH 10 DAYS PRECEDING SAMPLING		
	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN	MEAN TEMP.	SUN-SHINE	RAIN
	°*F.*	*per cent.*	*inches*	°*F.*	*per cent.*	*inches*	°*F.*	*per cent.*	*inches*	°*F.*	*per cent.*	*inches*
Series I:												
April 21	51.4	57.9	0.24	55.1	61.3	1.42	52.3	59.9	0.35	52.9	59.7	2.01
May 5	58.3	66.4	1.96	49.7	54.9	1.11	53.9	48.3	1.32	53.9	56.5	4.39
May 19	64.2	70.6	1.01	61.6	57.2	1.28	48.5	44.3	3.02	58.1	57.4	5.31
May 26	65.5	45.4	2.54	58.7	72.7	1.35	59.1	57.2	2.00	61.1	61.3	3.72
June 5	65.6	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
June 15	69.9	56.8	0.49	64.6	54.0	0.37	65.5	46.4	2.54	66.6	52.4	3.40
Series II:												
June 5	65.5	54.0	0.37	65.5	46.4	2.54	58.7	72.7	1.35	63.3	58.4	4.26
Series III:												
July 6	76.3	75.7	0.55	71.8	42.9	1.25	69.2	60.2	0.49	72.4	59.6	2.29
Series IV:												
Oct. 17	67.0	61.1	0.00	59.8	51.4	0.55	74.0	64.7	0.00	67.0	59.1	0.55

21 had only 0.24 inch rainfall, but the preceding 10 days had 1.42 inches. The period May 26 to June 15 had only 0.86 inch, but the preceding 10-day period had 2.54 inches. In no case during the taking of these samples was there a period of drought such that the soil became markedly deficient in moisture, nor was there any such marked or prolonged departure from normal in temperature or in incidence of sunshine as might be expected appreciably to affect the rate of development of the plants.

The third series, collected July 6, presents some contrasts with the second series. The mean temperature for the 30 days preceding July 6 was 9 degrees higher than for the corresponding period ending June 5, while the rainfall was only 54 per cent. of the amount for that period. These samples consequently developed in a period that combined high temperatures and resulting maximum transpiration with a considerable deficiency in water supply. The fourth series, collected October 17, developed in a period of markedly deficient precipitation. The 10 days immediately preceding sampling were entirely rainless, the second 10 days had 0.55 inch, and the third 10-day period was again rainless. The dry period had been of sufficient duration at the time of sampling to reduce soil moisture to a very low level, and the mean temperature for the month preceding sampling was only 5.4 degrees below that for the third series and 3.7 degrees above that of the second series.

Summarizing, the first and second series developed in a period of ample rainfall and low mean temperature; the third series developed in a period of subnormal rainfall and maximum mean temperature; the fourth series developed in a period of markedly deficient rainfall and intermediate mean temperature. The percentage of possible sunshine is markedly uniform in all the series. Obviously, any effect of environmental conditions apparent in comparing the several series may be expected to be that of progressive reduction of moisture content of the soil accompanying the progress of the season.

Analytical results

The results of the analyses are assembled in table III, and the changes in the several constituents are graphically presented in figures 1 to 5. The general course of the changes in composition of the petiole and leaf will be indicated, after which the details of differences between the several series will be discussed.

TOTAL SOLIDS

It is noted at once that there was a very striking difference in total solids between the mesophyll tissue and the petioles. The total solids of the leaf tissue were everywhere much greater, in the early stages about three times as great, as in the petiole. Total solids of the leaf decreased

TABLE III

.COMPOSITION OF RHUBARB PETIOLES AND LEAVES AT DIFFERENT STAGES OF DEVELOPMENT IN PERCENTAGE OF FRESH GREEN WEIGHT

DATE OF SAMPLING	AGE IN DAYS	SIZE OF LEAVES IN INCHES		TOTAL SOLIDS		SOLIDS SOLUBLE IN ALCOHOL		ALCOHOL-INSOLUBLE RESIDUE		TOTAL SUGAR	
		LENGTH PETIOLE	WIDTH LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF	PETIOLE	LEAF
Series I:											
April 21	10	2–4	3–4	5.21	15.49	2.96	5.64	2.25	9.85	0.65	0.74
May 5	24	10–12	8–10	4.80	12.97	2.44	4.52	2.36	8.45	0.30	0.62
May 19	38	12–14	10–12	5.71	10.02	2.80	3.08	2.91	6.94	0.42	0.42
May 26	45	14–18	12–14	5.87	9.71	2.90	3.32	2.97	6.39	0.60	0.47
June 5	55	14–18	12–14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56
June 15	65	14–18	12–14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48
Series II:											
June 5	5	3–4	3–4	5.33	16.40	2.84	5.60	2.49	10.80	0.40	0.89
" "	8	6–10	5–7	4.83	14.63	2.60	5.40	2.23	9.23	0.39	0.70
" "	11	10–12	6–8	4.60	14.59	2.48	4.84	2.12	9.75	0.33	0.60
" "	15	12–14	8–9	4.84	12.65	2.72	4.08	2.12	8.59	0.39	0.62
" "	19	14–16	8–10	4.56	13.27	2.56	4.16	2.00	9.11	0.29	0.40
" "	25	16–18	14–16	5.25	12.74	2.96	3.74	2.28	9.00	0.52	0.76
" "	32	18–21	14–16	5.81	11.17	3.16	3.28	2.65	7.89	0.60	0.65
" "	40	18–21	14–16	6.17	11.62	3.37	3.32	2.80	8.30	0.90	0.51
" "	55	14–18	12–14	5.81	9.70	2.96	3.12	2.85	6.58	0.67	0.56
" 15	65	14–18	12–14	6.52	9.54	2.96	2.72	3.56	6.82	0.66	0.48
Series III:											
July 6	5	4–6	3–4	6.57	18.07	3.44	5.80	3.13	12.27	0.40	0.79
" "	6	10–12	5–7	6.45	17.59	3.44	6.28	3.01	11.31	0.50	0.71
" "	13	12–16	7–10	6.15	15.82	3.20	5.40	2.95	10.32	0.60	0.61
" "	28	18–24	18–22	7.19	13.04	3.60	4.16	3.59	9.88	1.05	0.74
" "	42	20–28	17–22	7.43	12.37	3.48	3.44	3.95	8.93	0.91	0.52
" "	60	20–28	17–22	6.82	10.06	2.96	2.76	3.86	7.30	0.56	0.42
Series IV:											
Oct. 17	8	9–14	7–10	6.29	15.57	3.56	5.32	2.73	10.25	1.04	0.74
" "	12	14–18	10–12	6.35	15.02	3.44	5.32	2.91	9.70	1.04	0.66
" "	24	16–20	10–14	7.22	13.34	3.76	4.32	3.46	9.02	1.14	0.61
" "	40	16–20	10–14	7.23	13.33	3.84	4.24	3.39	9.09	1.05	1.36
" "	60	16–20	10–14	5.64	10.00	2.52	3.20	3.12	6.80	0.58	0.61
					ROOT						
April 21				13.14		5.96		7.18		1.76	
May 19				12.08		5.68		6.40		1.14	
July 6				22.09		6.76		15.33		2.44	
Oct. 17				24.44		6.40		18.04		1.69	

TABLE III—(*Continued*)

Acid-hydrolyzable polysaccharides		Acid as malic		Tannin		Nitrate nitrogen		Amino nitrogen		Total nitrogen	
Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf
0.55	0.90	0.999	0.441	0.096	1.08	0.014	0.002	0.045	0.042	0.211	0.943
0.53	0.79	0.948	0.688	0.058	0.432	0.030	0.004	0.029	0.039	0.203	0.824
0.67	0.69	0.884	0.595	0.086	0.277	0.067	0.007	0.027	0.032	0.178	0.560
0.68	0.59	1.000	0.611	0.109	0.326	0.065	0.007	0.036	0.038	0.155	0.409
0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
0.70	0.62	0.749	0.540	0.110	0.236	0.100	0.017	0.020	0.035	0.130	0.435
0.54	0.95	1.080	0.593	0.122	0.774	0.024	0.003	0.043	0.062	0.198	0.964
0.48	0.89	1.110	0.652	0.109	0.581	0.029	0.005	0.045	0.061	0.160	0.912
0.46	1.05	1.080	0.639	0.127	0.487	0.030	0.005	0.055	0.054	0.136	0.895
0.47	1.06	1.110	0.748	0.096	0.338	0.032	0.005	0.040	0.032	0.142	0.813
0.42	1.21	1.070	0.785	0.088	0.348	0.035	0.005	0.039	0.050	0.149	0.741
0.45	1.06	1.085	0.704	0.091	0.335	0.038	0.006	0.030	0.038	0.127	0.727
0.58	0.92	1.140	0.715	0.087	0.249	0.044	0.009	0.030	0.028	0.134	0.628
0.72	0.99	1.160	0.713	0.079	0.282	0.056	0.009	0.026	0.032	0.130	0.531
0.58	0.75	0.818	0.509	0.073	0.268	0.077	0.010	0.024	0.028	0.127	0.376
0.70	0.62	0.749	0.540	0.110	0.236	0.099	0.017	0.020	0.035	0.130	0.435
0.76	1.06	1.520	0.749	0.168	1.222	0.017	0.002	0.071	0.093	0.267	1.090
0.79	1.30	1.580	0.821	0.153	0.975	0.022	0.002	0.068	0.101	0.228	0.944
0.77	1.78	1.380	0.799	0.123	0.455	0.026	0.003	0.057	0.065	0.197	0.867
0.84	1.10	1.320	0.843	0.201	0.437	0.027	0.003	0.043	0.058	0.149	0.662
0.78	0.81	1.150	0.628	0.162	0.290	0.053	0.005	0.038	0.055	0.148	0.594
0.75	0.75	0.781	0.493	0.265	0.265	0.073	0.010	0.010	0.043	0.121	0.316
0.63	0.98	1.560	0.893	0.111	0.359	0.023	0.003	0.070	0.066	0.201	0.807
0.64	0.84	1.430	0.816	0.114	0.385	0.026	0.003	0.070	0.055	0.186	0.734
0.74	0.90	1.300	0.876	0.110	0.392	0.026	0.003	0.050	0.072	0.180	0.675
0.72	0.82	1.280	0.950	0.115	0.385	0.030	0.004	0.041	0.041	0.159	0.635
0.67	0.65	0.934	0.680	0.100	0.331	0.045	0.005	0.039	0.046	0.132	0.367
					Root						
2.24		0.362		1.260		0.0082		0.096		0.417	
1.91		0.717		1.410		0.027		0.069		0.386	
10.41		0.560		1.460		0.022		0.054		0.279	
12.60		0.576		1.500		0.026		0.062		0.447	

progressively with increasing age until the leaf had practically ceased to grow, then fell off more slowly until the leaf was senescent (fig. 1, curves *A′–D′*). The whole course of development of the leaf is accompanied by a progressive hydration which varies in rate with the amount of external water supply, as evidenced by the flatness of curve *D′* (representing a series grown under conditions of rather severe drought). It would appear that the developing leaf is high in its content of hydrophilic colloids and that these become progressively saturated as the leaf ages. With attainment of full size and cessation of the formation of new colloidal materials, absorption of water slows down, but continues, except in one case (figure 1, *A′*), up to the final sampling. It seems possible that the old leaf may die because it can no longer obtain water, lacking a saturation deficit of sufficient magnitude to maintain a water balance against transpiration.

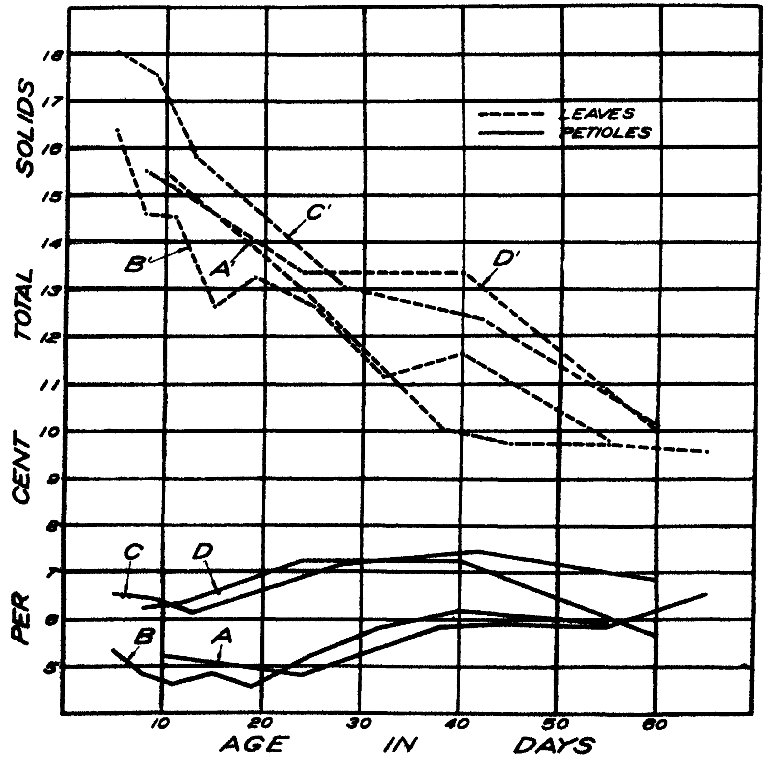

FIG. 1. Changes in total solids content in leaf blades and in petioles of rhubarb with increase in age and advance of season: *A*, *A′*, series collected April 21 to June 15; *B*, *B′*, series collected June 5; *C*, *C′*, series collected July 6; *D*, *D′*, series collected October 17.

The changes in total solids content of the petioles take a definite course which is of interest (fig. 1, *A–D*). During the period of rapid elongation of the petiole the solids decrease. As elongation slows down and the period of maximum photosynthetic activity of the leaf is entered upon, solids increase again, becoming stationary or falling off somewhat as senility of the leaf commences.

It will be observed that there is considerable difference in total solids content of both leaves and petioles in the series taken at different seasons. In series I and II, developing in the spring, total solids are consistently lower at all stages in both leaves and petioles than in series III and IV, collected in July and October. Further, series IV, collected in October, is distinctly higher in solids than series III, except in the old petioles. These differences would appear to be explained by the fact that the water supplied by rainfall during the last month of development of series III and IV was only about one-half and one-eighth that received in the corresponding period by series I and II. It would appear that the saturation deficit of the leaf tissues is largely satisfied in the first half of the life period if the water supplied the root is ample, but that its satisfaction is progressively retarded with progressive decrease in water supply.

Total solids of the root show a decrease from the first sampling, April 21, to that on May 19, due to depletion of the reserves of the root system by the developing foliage. On July 6, storage of reserves is well advanced, and on October 17 the total solids content of the roots is more than 200 per cent. of that present at the height of the growing period on May 19.

ALCOHOL-SOLUBLE SOLIDS

The alcohol-soluble solids of the leaf blade are high in the earliest samples taken and decrease rapidly until the leaf is full-grown, then more slowly up to old age. The final value in old leaves is about 50 per cent. of that of the youngest samples. In the petioles, soluble solids fluctuate within narrow limits, decreasing somewhat in the period of most rapid growth, then returning to the original level. In series III and IV there is a decrease in the final sample not found in the earlier series, for which no explanation is apparent.

The general level of alcohol-soluble solids is higher in the petioles of series III and IV, but there is no consistent difference in the leaves. The alcohol-soluble solids of the root show little change, decreasing slightly from April to May, then rising to somewhat higher levels in July and October.

ALCOHOL-INSOLUBLE SOLIDS

The alcohol-insoluble solids of the leaf blade decrease rather rapidly until the leaf is full-grown, then more slowly, with some fluctuations, to a value at final sampling about two-thirds of that found in the earliest sample. The alcohol-insoluble fraction in the petioles remains stationary or decreases during rapid growth, then rises somewhat with increasing age. The general level of insoluble solids in the petioles is a little higher in series III and IV than in the earlier series.

The fluctuations in both alcohol-soluble and alcohol-insoluble fractions in petioles and leaves of series I suggest that both may be temporarily affected by climatic conditions, and particularly by rainfall.

Alcohol-insoluble solids in the roots decrease to a minimum in May, when rapid development of aerial parts at the expense of stored reserves is occurring, then increase rapidly to the July sampling, and more slowly to October, when the amount is 282 per cent. of that present in mid-May. The large seasonal change in total solids of the roots is practically wholly due to the change in amount of the alcohol-insoluble fraction.

SUGARS

It is obvious that the total sugars are low in both leaf and petiole, and the differences between them are not marked. There is a slight tendency for the sugars of the leaf blades to be highest in young samples and lowest in old samples, while lowest in young petioles and higher in older ones; but between the two extremes there is considerable and rather abrupt fluctuation, so that the curves are irregular. It does not seem to be justifiable to draw any very definite conclusion as to the mechanism of the transportation and the use that is made of the sugars.

ACID-HYDROLYZABLE SUBSTANCES (POLYSACCHARIDES)[1]

The acid-hydrolyzable substances are very low in both the leaf and petiole. The figures indicate that the change in content with age is not great. They seem to be highest in both petioles and leaves in series III. In the series taken in June and July these substances are highest in the samples of medium age, being low when young and decreasing again with age. This is not apparent in the other two series. Starch is practically absent in the petiole and not high in the leaf. Acid-hydrolyzable substances are at a minimum in the root in May, increasing to over 600 per cent. of the spring value in October. Approximately one-half of the total solids of the October sample is in this fraction.

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[1] The term "acid-hydrolyzable polysaccharides" as applied to the substances converted to reducing sugars by the official method of direct acid hydrolysis for starch of the Association of Official Agricultural Chemists, which was here used, is technically inaccurate, since the reducing sugars obtained may be derived from polysaccharides, pentosans, glucosides, nucleic acids, or other substances having a sugar group in the molecule. While a misnomer, it has the merit that prolonged usage has made biochemists familiar with its real meaning as here employed. "Acid-hydrolyzable substances," while less restrictive, is indefinite unless the exact conditions of the hydrolysis are stated.

TANNIN

There is a striking difference in the tannin content of the leaf and petiole, that of the leaf being one and one-half to four times as high as that of the petiole. In the petiole the stage of maturity makes no striking difference until the very old samples are reached, when it becomes much lower. In the leaf the tannin is highest in the young and lowest in the old material. The decrease in amount in the leaf is rapid in the period of most rapid growth, 5 to 20 days of age, then becomes slow and fluctuates irregularly. Tannins in the root increase slightly from first to last sampling.

TITRATABLE ACIDITY

The titratable acidities of petioles and leaf blades show consistent and striking differences at all stages (fig. 2). That of the petiole is always much higher, being double that of the blades in the youngest samples and about one and one-half times that of the blades in the older ones. The changes

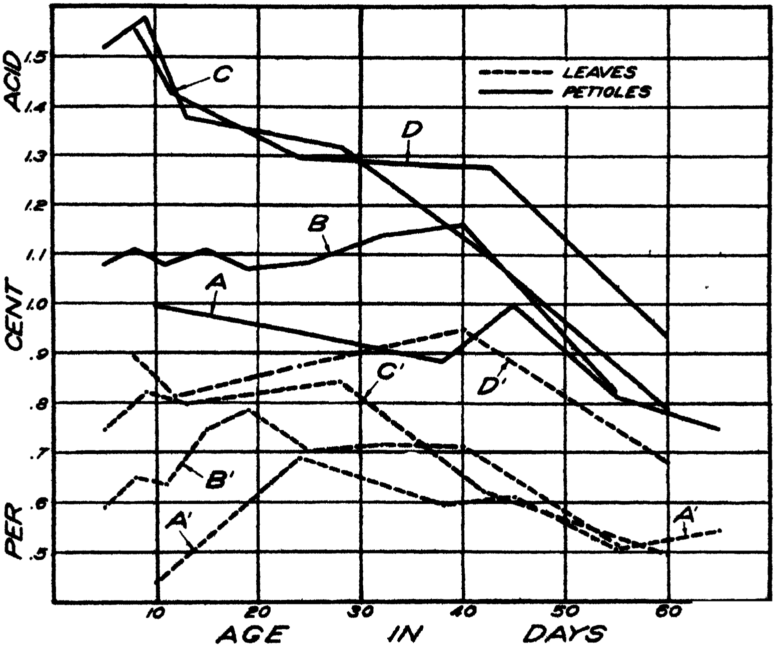

FIG. 2. Changes in titratable acidity of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

with age in the leaf tissue are an increase in the period of rapid growth, a period of little change during the period of maximum photosynthetic activity of the leaf, and a gradual decline as the leaf becomes old. In the petiole, acidity remains somewhat constant or decreases only slightly until the petioles are 45–55 days old, then declines rather rapidly in the old samples. It is noteworthy that the acidity of both petioles and blades of series III and IV is considerably higher throughout than in series I and II, that of the petioles being about 150 per cent. This difference may find its explanation in the reduction of available water supply during the development of the later series.

TOTAL NITROGEN

The curves representing total nitrogen content of petioles and leaves show the striking differences which exist (fig. 3). The total nitrogen of the leaf mesophyll is in the young leaf 400 to 500 per cent. of that of the petioles of like age; that of the old leaves is approximately 300 per cent. of that of old petioles. There is a marked decrease in total nitrogen of leaves with age, the final values for leaves 60 to 65 days old ranging from 35 to 45 per cent. of those of the youngest samples. The decrease is not especially rapid at any particular stage, but continues at a rather uniform rate throughout the whole life of the leaf. The petioles also decrease progressively and rather uniformly in total nitrogen content throughout life, the total decrease amounting to 30 to 50 per cent. of the initial content. The differences in level of total nitrogen content between the several series are not great in amount and are not correlated with the march of seasonal conditions.

The total nitrogen content of the roots decreases up to July 6, but has risen on October 17 to a level slightly above that of the initial sample of April 21. There is apparently a progressive depletion of total nitrogen in the roots so long as new leaves are being developed, followed by an increase after such development has ceased for the season.

There is clearly no such great and rapid reduction of total nitrogen in the old leaves by transfer into the perennial parts as has generally been found to occur in woody perennials and trees. The curve of decrease in the leaf is very nearly a straight line with no alteration in direction with old leaves, despite the fact that the blades of the final samples were generally yellowing and had dead areas at the edges. The present data do not permit decision as to whether the reduction in percentage of total nitrogen is only apparent and not actual, and attributable to the changes in composition occurring with age, or whether slight actual decrease occurs.

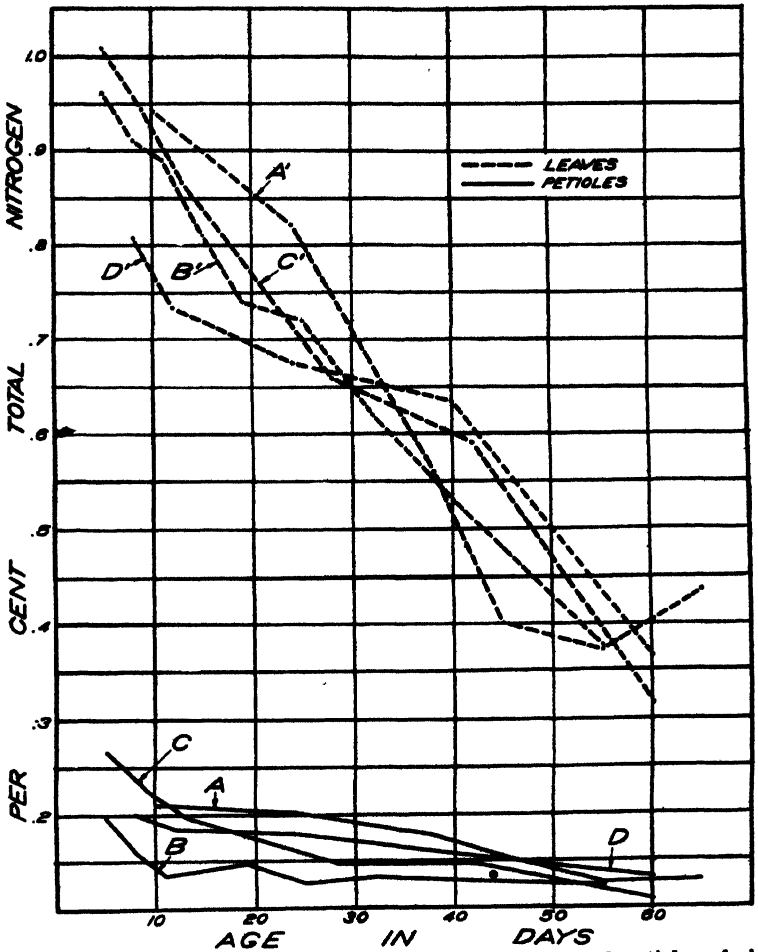

FIG. 3. Changes in total nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*. *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 6; *D*, *D'*, series collected October 17.

AMINO NITROGEN

The determinations of amino nitrogen were made upon material preserved in alcohol. They were completed prior to the appearance of the paper by WEBSTER (37) on the losses of amino nitrogen occurring in material preserved in this manner, especially when nitrates are present. It is very questionable whether the results have any quantitative value. The extremely irregular character of the curves is not conducive to confidence in their accuracy. The results are included in the table and represented in figure 4 for any comparative value they may have. They indicate that there is a progressive but rather irregular decrease in amino nitrogen from first to last samplings in both leaf blades and petioles. They seem to indicate also that there is a decided difference in general level of amino nitrogen content between the earlier and the later series, as series III and IV are

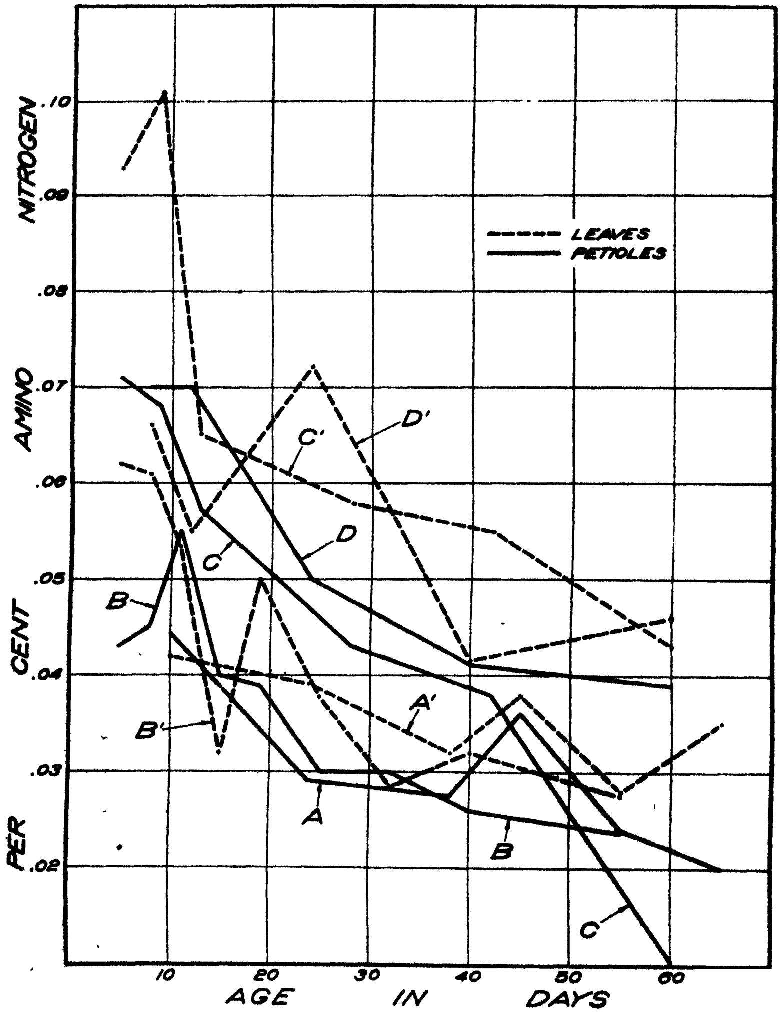

FIG. 4. Changes in amino nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A'*, series collected April 21 to June 15; *B*, *B'*, series collected June 5; *C*, *C'*, series collected July 5; *D*, *D'*, series collected October 17.

very materially higher throughout, both in petioles and leaves, than are series I and II. Whether the concurrence of higher amino nitrogen values in these series with the lower available water supply during their development is significant or merely a coincidence cannot be determined in view of the questionable character of the method employed.

NITRATE NITROGEN

As has been the case with several other constituents, and particularly with total nitrogen, there are striking differences between the leaf mesophyll and the petiole in nitrate nitrogen content. In contrast to total nitrogen, nitrate nitrogen is highest in the petioles, and the differences between petioles and leaves are greatest in the oldest material. In both petioles and leaves, nitrate nitrogen is minimum in amount in the youngest samples and rises with age (fig. 5). The amounts present in leaves are always low but the increases, while small, are consistent and significant, the final samples of the several series having amounts ranging from 160 to 850 per cent. of those of the initial samples of their respective series. The amounts initially present in the youngest petioles are much larger than in leaves, and increase rapidly with age, the final content of the old petioles ranging between six and nine times that of the old leaf tissues. It is apparent that the plant absorbs quantities of nitrate nitrogen far in excess of its anabolic requirements, and that the petioles serve as storage organs for the deposition of the excess. That this nitrate nitrogen is neither used in the leaf nor returned to the roots is clear, since the petiole attains its maximum nitrate nitrogen content when the leaf blade is yellowing and dying. The nitrate nitrogen content increases rather rapidly, even during the period of rapid growth when synthesis of protein is actively in progress, but becomes more rapid after the leaves are full-grown. There is, of course, a possibility that lack of some other constituent prevented utilization of the nitrate nitrogen and that it was consequently stored in the petioles, the metabolic processes never becoming such that it could be used. Further study will be necessary to determine whether development of flower stalks is accompanied by withdrawal of nitrate nitrogen from the petioles, since the variety employed is one which only exceptionally develops flower stalks, and none were developed during the progress of the work.

The progressive accumulation of nitrate nitrogen in the petioles is a matter of great practical importance in the canning of rhubarb, since nitrate nitrogen has an intense corrosive action upon the tin can. This matter has been dealt with in detail by CULPEPPER and MOON (**14**) in their report upon the behavior of rhubarb in canning.

It is unfortunate that soil samples were not taken and that it cannot be determined whether the amount of nitrate nitrogen in the tissues shows any

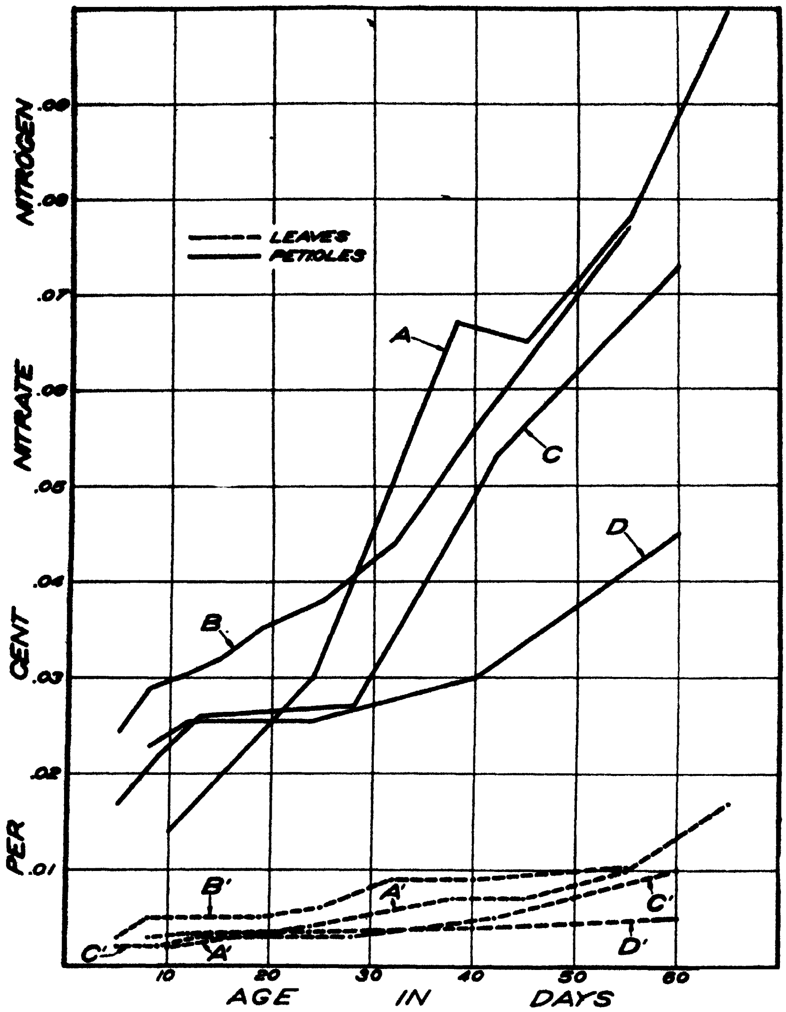

FIG. 5. Changes in nitrate nitrogen content of leaf blades and petioles of rhubarb with increase in age and advance of season: *A*, *A′*, series collected April 21 to June 15; *B*, *B′*, series collected June 5; *C*, *C′*, series collected July 6; *D*, *D′*, series collected October 17.

correlation with the amount present in the soil. It is probable that the reduction in amount of available soil nitrates with advance of the season may be responsible for the differences between the earlier series and series III and IV. In the first two series, nitrates are higher in both leaf blades and petioles than in the later series. Series III, taken in July, is consistently somewhat lower throughout in both leaves and petioles than I and

II while series IV is lower than III, the difference being especially marked in the old petioles. This may be a result of progressive decrease of available nitrate in the soil. Another fact which is significant in relation to the low nitrate content of series IV is the extraordinarily heavy rainfall of August, which had a total precipitation of 14.41 inches, or 10.40 inches' excess over the normal, largely occurring as heavy rains between August 11 and August 26. This excessive precipitation, which was followed by drought extending from mid-September through October, probably resulted in leaching out much of the nitrates present in the soil, and is without doubt a significant factor in the reduction of the nitrate content of the older samples of the October series to less than half that of the corresponding samples taken earlier in the season. That the reduction of available nitrate was limited in its effect to reducing the storage of excess nitrate in the petioles and did not affect the total nitrogen content of the leaves and petioles is immediately apparent from the graph (fig. 3, curves *D* and *D'*).

Discussion

The biochemistry of the rhubarb plant presents some interesting points of difference from that of woody perennials or that of annuals and biennials, which may be briefly summarized.

The work of LECLERC DU SABLON (**15**) with leaves of chestnut, pear, peach, quince, and willow trees and raspberries, and that of TUCKER and TOLLENS (**34**) with *Platanus occidentalis,* indicate that in these forms there is a progressive decrease in water content with age. A similar situation prevails in annuals, and is attributable in part to progressive increase in vascular elements and cutin and in part to the presence of such products of photosynthesis as starches and sugars. In the work of the investigators cited, as well as in all similar studies found in literature, the entire leaf and petiole were taken together, so that it is impossible to ascertain the nature of the changes taking place in the leaf mesophyll as distinguished from petiole, midrib, and veins.

In the rhubarb leaf from which petiole and larger veins have been separated, the situation with respect to water content is precisely the reverse of that recorded for entire leaves of other plants. The youngest material obtained had a minimum water content which increased steadily with advance in age up to senescence, but most rapidly during the period of rapid increase in size. In this respect the growth of the leaf blade of rhubarb bears less resemblance to that of other and entire leaves than it does to that of young fruits, which have been shown by CALDWELL (**11**) to be initially high in total solids and to increase markedly in water content during the phase of most rapid growth. The extent to which the situation found in the leaf blade actually differs from that in the mesophyll of other types of leaves can be known only when the changes in the leaf blade proper, separated from the petiole and principal veins, have been studied in such leaves. In one respect the rhubarb leaf differs markedly from those of most other dicotyledonous plants, namely, in the practical absence of stored starch at all times.

The carbohydrate economy of the rhubarb plant presents some interesting problems. The total sugar content of the leaf is always very low, reaching 1 per cent. of the fresh weight in only one sample. It is with one exception highest in the youngest leaf blades of the series, collected while still largely folded and partially covered by the soil, and consequently before active photosynthesis could have begun. The amount present falls off slightly during the period of most rapid growth, rises as the leaf becomes full-grown, and drops again in senescence; but the changes are within extremely narrow limits and could not be considered significant did they not occur in all the series. The sugar content of the petiole is also low at all stages of growth, being sometimes above and sometimes below that of the corresponding leaf blade. In the October series it is somewhat higher throughout than in the earlier series, but drops abruptly to the same general level in the final sample. The sugar content of the root system is practically the same on April 21 and October 17, but is lower in May and higher in July.

Starch is present only in traces in petiole and leaf, as determined by microchemical tests, and the total acid-hydrolyzable substances are always low. In the leaf the amount of such substances present in the young, partially folded blade is in most cases about as great as in the fully expanded photosynthetically active leaf, and the amount present in the old, yellowing leaves is two-thirds to three-fourths that present in the active leaves. The acid-hydrolyzable substances of the petiole are lower in amount than those of the leaf blades, and fluctuate irregularly without significant change from youngest to oldest samples. There is no evidence of an accumulation of starch either in leaf or petiole as a result of photosynthetic activity, and the results indicate that there is no accumulation of sugar as such in the leaf. The amounts of sugar in the leaves and their petioles vary independently in such fashion as to make it clear that there is no concentration gradient such as has been shown to exist in various other plants. While the samples were uniformly collected at 10 A. M. on clear days, when photosynthesis should have been actively in progress for several hours, the leaves and petioles of fully functional leaves show no consistently higher content of sugar than do the young, not yet expanded leaves or the old, yellowing ones. The manufacture and transfer of photosynthetic products is accomplished without any such accumulation of sugars in the leaf or in the conducting system as is a normal accompaniment of photosynthesis in foliar organs. The absence of evidence for such accumulation is the more remarkable in view of the rather high rate of increase in dry weight found by SACHS (**28**) in *Rheum*, by MÜLLER (**21**) in the related *Rumex*, and by BROWN (**10**) in *Polygonum* when the leaves were exposed to bright illumination.

The acid-hydrolyzable substances of the roots were low when first examined on April 21, at which time the largest leaves had petioles 2–4 inches in length and had only partially unfolded their blades. The content was slightly lower a month later. On July 6 it had increased to 545 per cent. and on October 17 to 659 per cent. of the amount found on May 19. The concurrent changes in sugar content of the roots are within rather narrow limits, and the variations in total solids agree closely in direction and amount with the changes in hydrolyzable substances.

In contrast with the low concentration of sugars in petiole and leaf and the absence of a concentration gradient in sugars from leaf blade to petiole, the acid content of the leaf mesophyll is always markedly below that of the petiole. Also, the acidity of both leaf blade and petiole increases to a maximum as the leaf attains full size and declines markedly as the leaf becomes senescent. In both blade and petiole the acid content at all stages of growth equals or exceeds the sugar content, and in the petioles is often two or three times as great. The acid content of the root system fluctuates considerably, being lowest in April, increasing 100 per cent. in the next month, and then declining to a nearly constant level from July through October. At all times the acidity of the roots is below that of the leaves and half or less than half that of the petioles. Since NELSON (**22**) found 1.77 per cent. malic, 0.41 per cent. citric, and 0.12 per cent. oxalic acid in the petioles, the results of the acidity determinations are reported as malic acid.

In the present work, only such oxalates as are soluble in both 80 per cent. alcohol and in water are included in the determinations of titratable acidity, as the titrations were made upon aliquots of the alcoholic extract after evaporating the alcohol and taking up in water. This method includes free acids and such soluble oxalates as those of potassium, sodium, and magnesium, but does not include calcium oxalate. According to ANGERHAUSEN (**1**, **2**), the oxalates of the leaf blade of rhubarb are largely water-soluble; those of the petiole practically wholly calcium oxalate. The data of table III indicate that total titratable acidity is at all times much higher in the petioles than in the leaf parenchyma, but give no indication as to the nature of the acid or acid salts present. No determinations of the total oxalate content of leaf blades, petioles, or roots were made.

The fact that the acid content of the young petiole and leaf is already rather high at a time when the leaves have barely emerged above ground and the blades have only partially expanded raises some interesting questions as to the rôle played by acids in the economy of the plant. CALDWELL (**11**) found that the period of most rapid growth in young fruits was also a period of rapid increase in both actual and titratable acidity, both of which culminated in maximum values about the time that the curve of growth passed into the self-retarding phase. His results indicate that in fruits, maximum acid production accompanies rapid cell division and increase in size, and a similar situation appears to exist in the young leaf.

Since the acid content of the mesophyll tissue is consistently lower than that of the petiole, the question arises whether acids are formed in the mesophyll directly from the products of photosynthesis and transferred into the petioles, or whether they are formed in the petioles as well as in the leaf tissue. STEINMANN (**32**) found that the titratable acidity of the mesophyll tissues of rhubarb increased slightly (10–15 per cent.) from apex to base of the leaf, while the increase in the veins from the smaller veinlets to the basal portion of the midrib was about 60 per cent., and a further slight increase was found in the petiole. These results STEINMANN considers as evidence that acids are formed in the leaf and transported to the roots through the petioles in the same manner as carbohydrates. The increase in acidity occurring in the leaf during a day's exposure to sunlight and the progressive decrease occurring in leaves kept in darkness in his experiments support his conclusion. His most conclusive proofs that acids are formed in the leaf and transported through the stem are derived from a series of experiments in which the petiole was cut half way through at the base of the leaf, thus severing the vessels of one-half the blade. After exposure to a day's sun, the acidity of the intact half of the leaf was always lower than that of the half with severed vessels; further, a reduction in acidity during darkness occurred in the intact half while no change occurred in the severed half. The transport of acids is consequently a constant process, in light as in darkness. STEINMANN also points out the possibility that organic acids may be not products of disintegration processes but intermediate products of constructive assimilatory processes. RUHLAND and WETZEL (**27**), on the other hand, consider that the progressive accumulation of acids and of ammonia and nitrate nitrogen in the plant with increasing age is the result of deamination of proteins.

ANGERHAUSEN (**1, 2**) states that the oxalic acid content of the leaf blades is approximately double that of the petioles, and that the oxalates of the leaf are predominantly potassium oxalate while calcium oxalate is predominant in the petiole. RUHLAND and WETZEL state that the youngest petioles contain only succinic and malic acid with scarcely a trace of oxalic, and that later in their development there is a sharp decrease in the former concurrently with rapid increase in oxalic acid. The work of VICKERY and PUCHER (36), and that of SMIRNOW, ERYGIN, DRBOGLOW, and MASCHKOWZEN (31), indicates that the situation in tobacco is the reverse of that in rhubarb, as oxalic acid is present in the young tobacco plant but decreases as development of the plant proceeds.

BERTHELOT and ANDRÉ (5) determined the oxalic acid content in roots, stems, petioles, and leaves of *Rumex acetosa* in young, actively growing and old, fruiting plants (June 26 and September 27). In the young plant the oxalic acid content of the leaf blades exceeded that of the petioles and larger veins in the ratio of 12 to 10; in the old plants the acid content of leaf blades was lower than that of petioles and stems, the ratio being 4 to 5. The acid of the leaf blades was predominantly in soluble form in both young and old plants; that of the petioles was predominantly soluble in young plants and chiefly insoluble in old ones. The acid of the roots was wholly in insoluble form. The relative amounts present in old petioles and leaf blades were 60 and 35 per cent. respectively of those in the young parts. The situation in *Rumex* consequently differs from that found in rhubarb in that the acidity of young leaf blades is greater and that of old leaf blades less than that of petioles, while the petioles of rhubarb are always much more acid.

At the close of their resumé of the literature on the origin of organic acids in leaf tissues, VICKERY and PUCHER (36) remark that the synthesis and transformation of the organic acids in higher plants are even more obscure than is the case in the molds and yeasts. It may also be said that in no part of the field is the obscurity greater than in that relating to the rôle of oxalic acid in green plants. No generalizations seem possible, since practically every fact established by the study of one plant is definitely contradicted by the findings in regard to another plant, as instanced in the preceding paragraph. In the case of rhubarb, it seems to be clearly established by the work of STEINMANN (32) that there is a continuous production of acid, assumed by him to be oxalic acid, in the leaf blade when the leaves are exposed to light; and that translocation of the acid through the veins into the petiole occurs both in light and darkness, so that there is a concentration gradient rising from a minimum at the leaf tip through the veins and midrib to a maximum in the middle of the petiole. That soluble oxalates are transformed into insoluble form in the petiole is evidenced by ANGERHAUSEN's results. Data here presented show that the maximum concentration of titratable acids found in leaf and petiole throughout life is found in the period of full photosynthetic efficiency, with lower concentrations in juvenile and senescent stages. These facts correlate the rate of production of acids with the general level of photosynthetic activity.

They leave the questions as to the mode of origin and rôle of oxalic acid in the plant unexplained. Whether it is a product of deamination of amino acids, as suggested by RUHLAND and WETZEL, or an intermediate product of photosynthesis playing a rôle in the metabolism of the plant analogous to that of simple sugars in other plants, as suggested by STEINMANN, or a waste product resulting from the operation of an insufficient respiratory mechanism which cannot carry the decomposition further, as the older literature implies, can be determined only by further research.

The course of the changes in nitrogenous constituents in the leaf is rather uniform in the several series. Total nitrogen and amino nitrogen are maximum in the youngest leaves and decrease rather steadily until the leaf is full-grown, after which amino nitrogen becomes fairly constant while total nitrogen undergoes further decrease until the leaf is senescent. There is rather close parallelism between the curves for the decrease in total solids and those for decrease in total nitrogen in the several series of leaf samples (figs. 1 and 4), which suggests that the reduction in amount of total nitrogen on the basis of reduction in percentage of fresh weight is due largely to dilution of the solids with water. In the initial samples of the several series, total nitrogen forms 5.8 to 6 per cent. of the total solids of the leaves; in the final samples it forms 3.15 to 4.5 per cent. of the solids. There is consequently no such large reduction in total nitrogen as SWART (**33**) and TUCKER and TOLLENS (**34**) found to occur in the leaves of *Platanus* and a number of other trees and perennial shrubs. In the case of *Platanus,* this reduction amounted to more than three-fourths of the total. The persistence of a relatively high content of total nitrogen in the old leaves does not disprove but certainly lends no support to the theory of RUHLAND and WETZEL (**27**), who attribute the formation of oxalic acid to the deamination of protein. Moreover, if the results for amino nitrogen are to be relied upon, its uniformly low concentration in petiole and leaf at all times would appear to militate against the possibility that deamination can be occurring at a rate sufficient to account for the large amount of oxalic acid and its salts present in the tissues.

A unique feature of the metabolism of the plant brought out by the present study is its remarkable capacity for absorbing and storing nitrates in amounts very far in excess of any subsequent needs. The work of WOO (**39**), CAMPBELL (**12**), and NIGHTINGALE (**23**) has made it evident that a wide variety of annuals and biennials, in their earlier stages of development, have high nitrate-absorbing capacity and store considerable amounts of nitrates in the aerial parts. Apparently high nitrate absorption is a function definitely limited to the early vegetative stages, since the plant becomes nitrate-free later in the season as flowers and seeds develop, and remains nitrate-free so long as it continues to live. In this respect, as in some others, the rhubarb plant presents a very different situation. The young and rapidly growing petioles and leaves are low in nitrate content, its utilization in the construction of protein apparently keeping pace with absorption and transport from the roots. But with the completion of growth of the leaf there is rapid increase in nitrate content of both leaf blade and petiole, but more especially in the latter, which continues without material slackening in rate until the leaf blades are yellowing and dying. Series of leaves developed early and late in the season, hence both before and after the usual period of development of the flower stalks, behave alike in this respect. Such differences in amount of nitrates stored as occur in the earlier and later series appear to be attributable to seasonal reduction in the soil nitrate supply and in available water rather than to any alteration in the capacity of the plant for absorbing and storing nitrates. That the nitrates so accumulated are never used in the petioles or translocated elsewhere is conclusively shown by the fact that the petioles of senescent leaves have maximum amounts, the total present in one case when calculated as molecular nitrogen reaching 1.53 per cent. of the dry weight of the plant. Furthermore, the course of the changes in nitrate content of the root system, which increases from April to May and then remains constant throughout the season, shows no relation to the changes in the petioles and leaves. The nitrates of the root are not depleted by the accumulations of nitrate in the petioles of the successive series of leaves, nor is there any indication that they are increased by translocation of nitrates out of the petioles as the leaves complete their life history and die. Lastly, there is obviously no appreciable utilization of the accumulated nitrates in the formation of amino acids or protein in the mature or aging petiole, since its content of total nitrogen decreases progressively from the unfolding of the blade to old age. There was in no case any development of flower stalks, so that we are without knowledge as to what would occur to the nitrates of the petioles in the course of development of flowers and seed.

Summary

1. The biochemical changes occurring in the roots, petioles, and leaf blades of the rhubarb plant in the course of the season's growth were followed by the collection and analysis of four series of samples, each consisting of subsamples of petioles and leaf blades of varying ages from 5 days onward to senescence, at intervals during the season. The first series consisted of the first leaves developed by the plants in spring, which were collected at intervals from April 21 to June 15; the later series was obtained from leaves developing in sequence and were taken on June 5, July 6, and October 17. Samples of the roots were taken on the same dates.

2. The seasonal march of climatological conditions during the period of development and collection of the samples exerted an appreciable influence upon the rate of development of the different series, the petioles and leaf blades of the later series attaining full size in a much shorter time than did those of series I and II. The chief environmental factor to which the more rapid development of the later series is probably attributable is higher mean temperature.

3. The general composition of the several series shows some differences which appear to be attributable to another environmental factor, namely, rainfall. Series I and II developed in a period of ample rainfall very equably distributed over the whole period of growth; series III developed in a period of considerably reduced rainfall; series IV developed in a period of well established drought, the total rainfall for 30 days preceding sampling being 0.55 inch. The reduction in precipitational water supply is reflected in the lower water content of both petioles and leaves at all stages of growth in series III, developed in a period in which transpiration was maximum. The effect of drought during development is also apparent in the leaves of series IV, which retained until senescence set in the high solids content characteristic of developing leaves.

4. Total solids content was maximum in the leaf blade at the time the blades were emerging from the soil and still partially folded, decreased rapidly during growth of the leaf, and became mostly stationary after growth ceased. In the petioles there was a decrease in total solids during rapid elongation, followed by an increase which brought the solids content of the old petioles somewhat above that of the young ones.

5. The alcohol-insoluble solids of the leaf blade decreased progressively throughout the life of the blade; those of the petiole were fairly constant until growth was completed, then increased somewhat in the old petiole. Alcohol-soluble solids of the leaf blade decreased rather steadily throughout life; those of the petiole decreased during rapid growth, then rose as growth was completed.

6. Leaf mesophyll and petiole were practically starch-free at all ages. The sugar content of both was always low, that of the leaf blade being usually highest before the blade had unfolded, decreasing somewhat during growth and fluctuating within narrow limits in the full-grown leaf. Sugar content of the petiole was generally minimum during rapid growth, rising slightly in the old petiole. The fluctuations in sugar concentration in petioles and blades are independent and there was no evidence of the existence of a concentration gradient such as would be expected if large amounts of sugar were transported from the leaf through the petiole.

7. The acid-hydrolyzable polysaccharides of the leaf in two series increased slightly up to attainment of full size; in the other series there was

a slow decrease from youngest to oldest leaves. The acid-hydrolyzable material of the petiole remained stationary or increased within very narrow limits. In both parts its concentration, like that of sugars, was always low.

8. The titratable acidity of the leaf mesophyll and petiole increased during rapid growth, then declined somewhat in the older stages. The acidity of the petioles was at all ages 150 to 250 per cent. that of the leaf blade. In some cases titratable acids, calculated as malic, made up 18 to 25 per cent. of the dry weight of the petiole.

9. The total nitrogen content of leaf blades and petioles declined gradually from youngest to oldest samples in every series. The reduction was relatively much less than that reported for the leaves of woody perennials and trees.

10. Nitrate nitrogen was present in extremely small amounts in the young leaves and increased considerably after growth was completed and the leaves became old. The youngest petioles had seven to eight times as much nitrates as the corresponding leaf blades and increased steadily to a maximum in the senescent petioles, which might have a nitrate nitrogen content equivalent to 1.53 per cent. of the dry weight. There was no indication that this nitrate is used or translocated elsewhere.

Division of Horticultural Crops and Diseases,
United States Department of Agriculture,
Washington, D. C.

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MECHANISM OF THE ACTION OF LIGHT AND OTHER FACTORS ON STOMATAL MOVEMENT

GEORGE W. SCARTH

Introduction

The factors which have been reported to influence stomatal movement are: (1) *light*; (2) *turgidity* of the leaf; (3) *temperature*; (4) physiological *shock*; and (5), under artificial conditions, certain *chemical agents.* On the efficacy of light and water supply there is general agreement, but the conditions of their respective dominance are not well understood and the mechanism of their action is still the subject of diverse theories. As regards temperature and shock, investigators differ on the question of whether these factors have any important effect at all on the stomata of plants in general.

With the possibility of all these agents interacting and interfering with one another in the experiments, it seemed desirable to make a broad survey of the action of all of them on the species worked with before prosecuting more intensive study of any one. The results of this preliminary survey are given in the present paper, as well as some more detailed work on the action of light. Further investigation on the effect of temperature is reported in a paper which will follow. Although these experiments have been chiefly confined to one species, *Zebrina pendula* (*Tradescantia zebrina*), occasional observation of other species has yielded no evidence that the physiology of *Zebrina* is in any fundamental way unique, although, of course, plants differ in their relative sensitivity to the respective factors. Since the object of the research was not to obtain quantitative data regarding the responses of the stomata of a particular species to environmental conditions but to investigate the mechanism of that response, which, like other deep-seated cellular processes, is probably little subject to specific differences, it seemed unnecessary to particularize in the title of this paper as to the species studied.

The changes in the stomatal apparatus which were principally followed may be enumerated at this stage, and some of their known relations pointed out.

The function of a stoma as a ventilatory organ is determined by the linear dimensions of its aperture. The *width of the slit,* therefore, was taken as the basic character with which the other variables were to be correlated. This was generally measured *in situ,* but sometimes after fixation in alcohol, after the method of LLOYD (**16**). The width of the opening depends upon the *shape* of the guard cells, which changes with their own volume and that of the adjoining cells. The *diameter of the whole stoma* was accordingly measured in some cases, as a further index of these morphological changes. The immediate dynamic causes of alterations in the shape and size of the guard cells are changes in certain turgor pressures: first and foremost is that of the guard cells themselves (because, as several workers have shown, this changes to a much greater degree than the following): secondly, is that of the subsidiary cells and, in diminishing degree, of more remote cells. As to the rôle of the neighboring cells, the shape of the guard cells is modified considerably by the mechanical pressure of the flanking cells but, on account of longitudinal ridges in their own walls, little if any by those cells which abut on their ends. We may say, therefore, with URSPRUNG and BLUM, that it is the difference between the turgor pressure of the guard cells and of these subsidiary cells which determines the stomatal aperture. At equilibrium these turgor pressures vary as the osmotic pressure or ''osmotic value'' of the respective cells. It was enough for the purpose of this study to take the *osmotic value at incipient plasmolysis* as a standard of comparison. This was done frequently for guard cells and sometimes for the subsidiary cells also, using $CaCl_2$ or cane sugar solutions. When equilibrium does not exist, that is, when the suction pressure of the guard cells and subsidiary cells respectively is not equal, their turgor pressures are determined in part also by the external forces which bring about this inequality. Owing to their position and the cuticularization of their free walls, the guard cells seem both to gain and to lose water by way of the subsidiary cells. At any rate, a hypertonic solution applied to a section withdraws water from and reduces turgor in the subsidiary cells more rapidly than in the guard cells, causing temporary opening of stomata. The same has been reported frequently as the initial effect of wilting. Similarly water, whether applied to a section or injected into a leaf, tends at first to promote closure. For this and other reasons, in all the experiments except those specifically directed to test the effect of wilting, the leaves have been kept turgid.

Effects of reduced turgor will be further described in the section devoted to that topic.

Going a step farther back to the physico-chemical mechanisms of the osmotic changes, it is well known that in the guard cells reversible transformation of carbohydrate from sugar to starch and back again is of major importance, at least in the diurnal cycle; but nothing is definitely known as to whether addition and removal of carbohydrate may also play a part. If and when this happens, we should expect to find that the osmotic value and the amount of starch do not vary reciprocally. *Changes in the starch content* of the guard cells were therefore estimated in most of the work.

This was done by comparing the size of the starch grains after decolorizing with alcohol and staining with iodine in KI.

In a former paper (**22**), some evidence was presented of turgor being determined to some extent by other and more rapid processes, including, it was suggested, changes in the hydration capacity of colloidal material in the cell sap. This aspect of the problem was not pursued in the present study because, in the course of any major movement, such change, if it is a fact, is always accompanied or followed by the more easily observed hydrolysis or synthesis of starch, and my principal object was the investigation of the more fundamental processes through which both the osmotic and colloidal changes are linked with the external factors that govern them.

In the same paper (**22**) it was stated that guard cells stain differently with certain indicators, according as the stomata are open (in light) or closed (in darkness). The results point to an increase of pH in light. On account of the many difficulties and sources of error attending this kind of work, these experiments were repeated and extended. In addition, similar tests were applied in relation to the action of other factors than light. The technique of these indicator tests will be described in the section on the effects of light.

The various factors are considered separately, in the following order: (1) shock and wound stimulus; (2) temperature; (3) turgidity of the leaves; (4) light; (5) chemical agents which affect the pH of the guard cells.

I. Effects of shock and wound stimulus

Before we can satisfactorily study the action of environmental factors on stomatal movement, it is necessary to take stock of any possible effect of the mere manipulation of the material in the process of experimentation. Mechanical stimulation of the plant or leaves by shaking, rubbing, exposing to blasts of air, etc., which was found by KNIGHT (**10**) to induce partial and temporary closure in certain plants, seems to have no effect on *Zebrina pendula* or (so far as observed) on any other species employed in the present work. Sudden pronounced changes of temperature produced slight temporary movements, as will be described later, which may fall into the category of "shock" effects. More important movements are produced by actual injury of a portion of the leaf. If, for example, by touching with a hot glass rod, by applying a drop of strong acid, or by cutting with a razor, some of the cells are killed, stomata in the immediate vicinity of these cells tend to close. No effect is noticeable more than a millimeter or so away from the actual wound; but since sectioning is so commonly used to expose cells for observation and treatment, it is very important to discover what changes in their properties are produced by the operation, and to distinguish between the effect of the stimulus transmitted from the wounded cells and the change of physical environment to which cells in the neighborhood of a cut are subjected.

EXPERIMENTAL RESULTS.—Sections from the lower surface of a leaf of *Zebrina,* and also whole leaves of which the uncuticularized cell walls of portions of the lower epidermis were exposed by shaving off the overlying tissue, were placed in various environments and the behavior of stomata thus "exposed" was compared with that of normally "covered" stomata. The experiment was usually begun with open stomata and the preparations were kept in the light, so that the "covered" stomata remained practically unchanged or, if the experiment was prolonged overnight, they underwent normal changes in illumination. When the preparation is immersed in water, the "exposed" stomata close rapidly in spite of continued illumination. Visible closure begins within a few minutes and may be complete in some of the stomata within 15 minutes—a much shorter period than is required for ordinary closure induced by darkness or wilting. This applies to stomata with intact subsidiary cells. If the latter are destroyed by the cutting, so that their pressure on the guard cells is removed, the stomata immediately widen and do not close later; but the same internal changes take place in these guard cells as in those of intact stomata. As regards the latter, the rate and degree of closure decrease with their distance from the cut surface, and movement is not appreciable beyond the thickness of a few cells.

In water freed of CO_2 but otherwise aerated, the early behavior is exactly the same as in tap water. The closure, therefore, is not due to the slight increase in the CO_2 concentration of the environment.

In moist air or in oil (nujol) the exposed stomata still close, but less rapidly, and, on the whole, to a less degree. Evidently the presence of free water, temporarily at least, promotes closure. This effect of water is also seen when uncut leaves are infiltrated with water. Stomata over water-filled stomatal chambers do not, as a rule, open quite so widely as over air-filled chambers. No doubt the subsidiary cells and other parts of the epidermis are usually unsaturated and become more turgid in presence of water; while, relatively, the guard cells either take up water more slowly or in less degree.

The fact that even in oil or air the stomata near a cut surface quickly close, however, indicates that changes in tissue tension are not the sole or main cause of the movement. This is proved more directly by a study of the osmotic properties of the guard cells. The osmotic value at incipient plasmolysis was observed to fall markedly as closure proceeds. In one case, for example, the guard cells of "covered" stomata were plasmolyzed in $M \div 3$ but not in $M \div 4$ $CaCl_2$, whereas in the same preparation "exposed" guard cells half an hour after cutting were mostly hypotonic to M ÷ 6 $CaCl_2$. Little increase in starch can be observed while the osmotic value is falling, at least for half an hour or so. It is possible, of course, that the sugar which is abundant in open stomata is converted into some osmotically inactive carbohydrate other than starch, but the speed with which the osmotic pressure is reduced and the persistence of low pressure long after the actual physiological shock effect should have worn off point rather to a temporary increase of permeability and loss of solute from the guard cells than to a reversible condensation of carbohydrate within them. On the day after cutting, the "exposed" guard cells still fail to open in light and their osmotic value remains low. Sometimes also their starch content is now high, compared with that of "covered" guard cells in the same preparation. This starch increase was not observed in water free of CO_2 and may be the result of pH difference, as explained later.

From these experiments it is obvious that we cannot arrive at a knowledge of the behavior of stomata under normal conditions from a study simply of those near a cut surface. Fortunately, however, the reagents usually penetrate beyond the range of the more serious effects of the injury and they can also be introduced into whole leaves without having resource to cutting. These precautions are particularly necessary in staining with indicators. As regards the solutions ($CaCl_2$ and cane sugar) used in estimating osmotic value, it would seem that, if applied immediately after cutting, they counteract in large measure the injury effect, presumably inhibiting the increase of permeability; otherwise there would not be, such high osmotic values in the guard cells of open stomata. These high values are evidently not due to penetration of the reagents, because there is no subsequent deplasmolysis in either $CaCl_2$ or cane sugar. On the other hand, there *is* a general fall in the osmotic value of guard cells initially open, when these remain for hours in the solutions mentioned; but this is accompanied by increase of starch and is probably due to condensation rather than to exosmosis of sugars.

No pronounced change in the osmotic value of the epidermal cells was observed to result from sectioning. It would seem, therefore, that the fall in turgor as a response to injury of neighboring tissue is more or less specific to guard cells and particularly to those of open stomata.

In a paper published several years ago, Arends[1] has recorded the rapid closing of stomata of *Zebrina* in the vicinity of a wound. In one important point, however, my observations fail to agree with Arends' and repetition of the experiment does not alter my opinion. Arends says that starch is rapidly formed in the guard cells as the stomata close, reaching its maximum in 20 minutes, after which time no further condensation can be observed. I find, on the other hand, that if starch is absent to begin with, then whether in light or darkness, no more than mere traces can be detected after 20 minutes, even in the guard cells of stomata which have closed completely; moreover the starch gradually increases in amount for several hours after wounding. When the wound response was first observed, I was particularly watchful for starch production, because the hypothesis was entertained that closure might be caused by "acid of injury" developing in the guard cells or neighbouring cells, the production of which in guard cells near a wound is suggested by the reaction with indicators, as described later. But whereas the rate of starch condensation after wounding is of the same order as in intact leaves with open stomata when subjected to acetic acid vapor, the closing movement is much more rapid than when produced by acid, darkness, or wilting; and complete closure is attained with much less (if any) starch formation than under these other conditions.

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[1] Arends, J. Ueber den Einfluss chemischer Agenzien auf Stärkegehalt und osmotischen Wert der Spaltöffnungschliesszellen. Planta 1: 84–115. 1925.

The possibility of temporary accumulation of some other osmotically inactive carbohydrate than starch has already been mentioned, as well as my reasons for thinking that this is not sufficient explanation of the anomaly, and that escape of solute is at least a part of the mechanism of the drop in turgor.

It may be added that closure of stomata near a wound has been observed in many species of plants besides *Zebrina.*

II. Effects of temperature: preliminary experiments

Many investigators have found that stomata tend to open with increase of temperature; many others that they do not; and others again that the behavior is variable. In a few preliminary experiments with detached leaves of *Zebrina* kept in water, the writer found that high temperatures (38° to 40° C.) not only accentuate stomatal opening in light but tend to cause it or prevent closure, as the case may be, in darkness; while low temperatures (0° to 8° C.) prevent opening in light. Moreover, when some of the guard cells in such leaves are stained with methyl or ethyl red, as described in the next section, the quality of the color varies in such a way as to indicate that the pH of the cell sap increases with rise of temperature.

These experiments showed the necessity of temperature control in testing the effects of other factors. Accordingly, in the experiments which follow on the action of light, this control was practised whenever the light was sufficiently intense to make it necessary.

More systematic study of the effects of temperature on the properties of the guard cells was carried on with the help of a colleague and will be reported in a paper which will follow. This later work confirms the pH change and shows that it is accompanied by the same transformation of carbohydrate as in the response to light.

III. Effects of leaf turgor

The relation of stomatal opening to the amount of water in the leaf is a very complex one. Whether turgidity be above or below normal, the result varies with the duration of the abnormal state. It also varies considerably with the species of plant and the age and position of the leaves, while, of course, it is modified by the other external factors which regulate opening (BURGERSTEIN 1).

As regards the time factor, three phases have been distinguished in the process of wilting. (1) There is considerable evidence of an opening movement in the early stages, in certain plants at least—although this has been observed directly in only a few cases, *e.g.*, by STALFELT (**23**) and SAYRE (**20**). The initial increase of transpiration observed by DARWIN (**2**); and even the rise in porometer readings of LAIDLAW and KNIGHT (**14**) may conceivably be due to non-stomatal regulation, as argued by LLOYD, who was unable to observe any initial opening of stomata during wilting. Apparently plants differ in this respect. (2) More pronounced wilting results in closure in the majority of plants. According to various workers, however, a considerable percentage show open stomata. BURGERSTEIN, who investigated a great number of species in this regard, found that 16 per cent. of the herbaceous plants and 10 per cent. of the woody plants examined showed wide open stomata in the wilted state; while 40 per cent. herbaceous and 23 per cent. woody plants showed only partial closure. Such data, however, ought to be considered in relation to light and temperature as well as to water supply. The reduction of transpiration which automatically accompanies wilting may result in a rise in temperature of the leaves, if they are exposed to direct insolation. (3) In an extremely dry and, as BURGERSTEIN believes, posthumous state of the leaves, stomata which were previously closed may open.

The early opening is probably a direct effect of the reduction in the amount of water in and pressure of the neighboring cells; the secondary closure is certainly an indirect effect which consists, as shown by ILJIN (**6**) and STEINBERGER (**25**), in a reduction of the osmotic pressure of the guard cells through transformation of sugar to starch, just as in darkness; the final opening, when it occurs, may again be a direct action, namely, the result of tissue shrinkage.

Usually high turgidity, like low, brings a chain of changes rather than a single response. Infiltration of the leaves of *Zebrina* with water tends to reduce the stomatal aperture slightly. This is a direct and almost immediate effect, but a high state of turgor also has a less immediate indirect effect. It favors starch hydrolysis in the guard cells and stomatal opening in light, as has frequently been observed, and also induces it in darkness, as the writer's next paper will show.

A few personal observations may be added to the preceding statement of the effects of wilting. In the first place, it is very noticeable that stomata and even guard cells respond individually and not *en masse.* Some stomata may become completely shut before others adjacent to them are scarcely contracted, and the inner edge of one guard cell of a pair may be straight while the other is still half bent. In the second place, *the guard cells of wilted leaves exposed to the light stain with methyl and ethyl red like those of leaves kept in the dark and not like those of turgid leaves in the light;* that is to say, they stain pink with a dye solution which appears practically colorless in illuminated turgid leaves. This result was obtained when sections from the respective leaves were placed for a short time in the stains, but a similar result was obtained when leaves infiltrated with and partially dehydrated by a sugar solution containing dye were compared with leaves infiltrated with an aqueous dye solution after both had been exposed for some time to light. This staining distinction points to a difference in pH of the cell sap in turgid and wilted leaves in the light. In later experiments, to be reported in the next paper, a similar distinction was noted in the case of leaves kept in the dark. The principal point to be noted is that the major movements of stomata in response to changes of leaf turgor are accompanied (1) by starch-sugar transformation in the guard cells (as is already known) and (2) by staining differences which indicate a change of pH.

What significance these observations may have on the mechanism by which the water relations of the leaf regulate the osmotic value of the guard cells can be discussed more conveniently in the light of experiments which follow.

IV. Effects of light

A picture of the series of events which takes place in the guard cells under the influence of light was adumbrated in the work of DARWIN (**2**) and ROSING (**19**), and more clearly delineated by LLOYD (**16**), ILJIN (**6**), HAGEN (**5**), and others. They are chiefly disappearance of starch and increase of sugar from which ensues the osmotic intake of water that causes opening. Other light effects, first noted by WEBER, are increased resistance to heat, decreased adhesion of the protoplast to the wall, cessation of plasma streaming, a more scattered distribution of the chloroplasts, and changes in the form and visibility of the nucleus and nucleolus. These changes are probably incidental and not directly concerned with the opening mechanism, but any theory of that mechanism should account for them also.

KISSELEW (9) and NIKOLIC (18) argue that the speed with which stomata sometimes change their posture, especially in the direction of closure, points to a change of permeability rather than osmotic pressure. My results, already described, on the effect of treatments which may be regarded as causing "shock," agree with this; but the ordinary photic response (or by far the greater part of it at least) is gradual and proceeds *pari passu* with change in the osmotic value of the guard cells not to be accounted for in terms of permeability.

Perhaps this statement may require modification if the progress of movement is minutely analyzed. Fluctuations in the earlier stages of opening have been recorded by NIKOLIC (18) and STALFELT (23). On the basis of relation to the light stimulus, STALFELT distinguishes the "stretching phase" of opening up to the first appearance of an aperture and the "motor phase" from there onward. The progress of the second depends quantitatively on the amount of light received, *i.e.*, on the intensity × time (beyond a certain minimum intensity), while that of the first stage does not so depend. It is also fluctuating, but so (according to STALFELT) is the movement in darkness in leaves and shoots which have been removed from the plant. In view of the rather complex adjustments of suction tension and turgor between the guard cells and subsidiary cells, which follow any change in the relative rate of gain and loss of water by the leaves, the apparent periodicity of this response does not necessarily imply that it fails to fit into any of the categories which we are considering. At all events, the present study deals with only the major trends of movement and with the links between these and the environmental changes to which they are due.

Another effect of light on the guard cells, which was previously reported by the writer and for which the evidence is now fully presented in this paper, is a modification of their staining with certain indicators, pointing to an increase in the vacuolar pH. That this change may be essential to the mechanism rather than merely incidental was suggested by the similarity of the results produced through artificially varying the acidity of the guard cells to those produced normally by changes in illumination. In an earlier paper (22) it was shown that when epidermal sections of a leaf are kept for some time in a dilute solution of ammonia, even in the dark, starch disappears from the guard cells and the stomata tend to open, while dilute acetic acid, even in the light, produces the opposite effect. Moreover, the subsidiary changes just mentioned were in large measure duplicated under this treatment. On the basis of these results, the writer went so far as to put forward the working hypothesis that the ordinary effects of light and darkness respectively act through the intermedium of a pH change, as determined probably by the relative rate of assimilation and production of CO_2 —and possibly other acid products of respiration. To test this hypothesis, further experiments have been carried out on the relation of stomatal behavior to light and to hydrogen-ion changes.

The principal points of the various experiments will be clearer if they are grouped according to the problem toward the solution of which they were designed to contribute.

(1) *Are the changes in staining property of the guard cells which are induced by changes in illumination really indicative of a shift in the pH of their cell sap?* Under strictly controlled conditions, in which the leaves and indicator solutions and all environmental factors, except light, are invariable, the guard cells of *Zebrina pendula* (and other plants tested), if they stain at all with methyl red, ethyl red, or propyl red, always show differences of staining in relation to whether the leaves have been exposed to light or darkness. Sometimes this difference amounts only to a more rapid absorption of dye in leaves taken from the dark and deeper staining in a given time or to uncertain differences in tint; but often the color distinction is qualitative and clear cut, guard cells from the dark appearing pink and from the light colorless. The latter immediately become equally pink when treated with acetic acid, which proves that in lighted guard cells the indicator is present but on the alkaline side of the point of virage.

This color difference in the staining of the guard cells can be observed in sections which are quickly stained and examined after being taken from the variously treated leaves. They can also be seen in thick sections which are first stained and then exposed to light or dark respectively. They are more pronounced in leaves which have been partly infiltrated with dye solution, either before or after the treatment as regards light. The distinction is not dependent upon whether the stomata are closed or open because, for example, on exposure of leaves to light the indicator reaction begins to change *before* the stomata open or when only a slight slit is showing.

Having observed these differences hundreds of times, the writer is convinced that they are not accidental, although the result may easily be upset by a difference in the type of leaf or in the reaction of the indicator solutions, as already pointed out. The simplest explanation of transformation of the indicator from red to yellow or yellow to red is a change in H-ion concentration of the cell sap. Even if the property of binding the stain in its red state is imparted by the production of a negatively charged colloid, such a colloid is likely to be an acidoid, *i.e.*, one which dissociates H-ions into the liquid phase.

(2) *What appears to be the range of the pH of the guard cells and other tissues as affected by light and darkness respectively?* Whole plants or detached leaves placed in water were kept in the dark for several hours and respectively exposed to the light until their stomata were well open. The leaves were then infiltrated with various indicators, and after 10 minutes

or so they were washed and the under surface and sections from it observed under the microscope. The color of the indicator in the intercellular spaces underlying the stomata was noted in the intact leaf and the color in the guard cells observed either in the intact leaf or in sections. Guard cells and intercellular spaces vary together. The most acid reaction observed in the guard cells was red with methyl red, but yellow with benzene red (*i.e.*, apparently between pH 4.8 and 5.0). The most alkaline reaction was purple with brom cresol purple (the only acidic indicator that could be detected in the guard cells), but red with neutral red (*i.e.*, apparently between pH 6.0 and 7.0).

There is possibility of considerable error in the turning point of neutral red, which is very strongly absorbed by the cells and does not change to yellow very readily when weak ammonia is applied. The fact, however, that the flavone pigment naturally present in guard cells, as well as in the other epidermal cells, does not become yellow in the light indicates that the pH does not extend at any rate beyond pH 7.4, up to which point the flavone is colorless. (The turning point of the flavone was determined by extracting the mixture of anthocyanin and flavone pigments which are present in the epidermis of *Zebrina* and finding the pH points at which the mixed solution underwent color changes. The solution is reddish-purple (as in the leaf) at pH 7.0, changes to bluish at about pH 7.2 and to green at about pH 7.4 to 7.5. The green is due to the addition of the yellow of the flavone to the blue of the anthocyanin, so that up to about 7.4 the flavone evidently remains colorless.)

Unfortunately the writer has no basic nor amphoteric indicator with range between pH 6.0 and 7.0 to discover more readily the most alkaline limit of the reaction attained by the guard cells of *Zebrina* and *Tradescantia*. However, in *Pelargonium* leaves exposed to sunlight, neutral red in the guard cells sometimes has a brick red or orange hue. differing from its pink shade in the other cells and indicative of an almost neutral pH.

As regards the pH range of the liquid in the infiltrated leaf spaces, the most acid reaction—observed once or twice in *Zebrina*—was greenish with brom cresol green (turning blue with ammonia) and reddish with methyl red; *i.e.*, around pH 4.0. The most alkaline reaction was blue with brom thymol blue; *i.e.*, over pH 6.6. Brom cresol purple has a useful range (pH 5.8 to 6.2) for qualitative demonstration of pH change in the leaf spaces. The color of this indicator in the stomatal chambers can be changed to and fro from purple to yellow, according as the infiltrated leaves (lying in a water bath) are kept in light or darkness. (The yellow, as with all these dyes, is practically invisible as observed under these conditions.)

What partial pressure of CO_2 is necessary to change the particular dye solution used to the most acid reaction observed in the intercellular spaces, a pH of 4.2 or less, was not determined. With distilled water, 20 per cent. CO_2 would be sufficient to give a pH of about 4.0, but in the solution the buffer action of the dye would require a higher concentration of CO_2 to produce the same effect. Twenty per cent. CO_2 has been measured in potatoes. In potato sap 20 per cent. CO_2 produces a pH of only about 6.0; but guard cells, in view of their natural fluctuations of pH, are probably much less buffered than potato cells.

As regards the cells of the leaf other than the guard cells, no definite results were obtained as to their pH or changes therein. The indications are that it is fairly constant. Acidic indicators, in particular brom cresol purple, were allowed to accumulate in the leaf cells of *Anthericum* by setting cut leaves in a solution of dye, but no appreciable difference in coloration was noted according as the leaves were kept in light or darkness.

To summarize, the effect of light *vs.* darkness on the indicator reactions in the leaves is as follows: (a) In the intercellular spaces, *i.e.*, in a concentrated solution of the indicators in equilibrium with the internal atmosphere of the leaf, there is an apparent range from over pH 6.6 in light to about pH 4.0 in darkness. (b) In the leaf cells in general there is no great change so far as could be determined from this incomplete study. (c) In the guard cells there is a wide change from between pH 6.0 and 7.4 in light to less than pH 5.0 in the dark.

These results demonstrate a correlation between the concentration of CO_2 in the leaf spaces, the pH of the guard cells, and the osmotic changes within them. So far as I have gone at present, however, they do not prove a causal relation between these phenomena. The photosynthetic action of light and its stimulation of the guard cells may be entirely independent. The relation between these two actions is the next subject of inquiry.

(3) *What relation does the effect of light on stomata bear to its photosynthetic activity?* Two lines of investigation were followed: (a) the effect of light with respect to its wave-length and its absorption by chlorophyll; and (b) the effect of light on stomata separated from the green tissue of the leaf.

a. *Effect of light with respect to wave-length:* KOHL (**11**) found that of the visible spectrum only the region between the *B* and *C* lines, that is the red, and between *F* and the violet, that is the blue, were effective. LLOYD (**16**) found the blue to be less effective than the red. KOHL showed that infra-red radiation also causes opening, but, as later experiments will show, this may be a temperature effect.

In the experiments which follow, regarding the action of light of different wave-lengths on the pH as well as the posture of the guard cells, temperature has been kept constant. Dishes containing detached leaves were set in running water and with a water screen between them and the source of light—a 150-watt Mazda lamp placed about 6 inches above the preparation. The dishes, each containing two leaves, were covered respectively by the following filters: a ground glass screen, an opaque screen, and two Wratten and Wainwright filters, allowing only red, green, and blue light to pass. The leaves, taken from plants which had been kept in the dark overnight or longer, were slit in numerous places and centrifuged for about two minutes in saturated ethyl red; about 10 minutes later, by which time guard cells near the slits were stained pink, the leaves, still lying in dye solution, were exposed as described to various types of illumination.

After an hour or more the stomata always opened somewhat (slightly to half open) under both white and red screens, and the stain in the guard cells faded greatly or sometimes disappeared; *i.e.*, became yellow. In darkness the stomata remained shut and the stain intensified. Under the green and blue screens the result was sometimes indistinguishable from that of complete darkness, although at other times there was slight opening and reduction of stain. To obtain pure blue light, two Wratten and Wainwright screens had to be employed and the intensity of the light transmitted as very small. There was more evidence of activity with the Corning blue glass screen no. 10. The intensity as well as the quality of the transmitted light would have to be regulated in order that the relative efficiency of the spectral regions might be determined, but this analytical mode of investigation was not pursued very far because it seemed possible to arrive at the information required more simply and directly by using chlorophyll itself as the color screen. It also seemed better to use rooted plants than detached leaves, since fuller and more regular opening of the stomata takes place in the former.

Potted plants well supplied with water were placed in earthenware crocks and covered by dishes containing liquids with different light-absorbing qualities: (1) India ink; (2) chlorophyll in alcoholic solution; (3) chlorophyll with Cu substituted for Mg; (4) solutions of red dyes, such as basic fuchsin and safranin, which transmit the red band absorbed by chlorophyll. The chlorophyll was changed every half-hour or hour, or at longer intervals (depending on light intensity) before any sign of decomposition or colloidal precipitation appeared. The preparations were simultaneously exposed either to diffuse daylight or in some cases to direct sunlight. It was found that a clear transparent chlorophyll solution may completely inhibit the light activity under conditions of illumination in which an India ink solution two or three times as opaque to the eye and basic fuchsin or safranin of equal opacity allow full and normally rapid opening. (Stomata of *Zebrina* open well in very weak daylight.)

Although the only measure of intensity used was brightness to the eye, the difference between the effect of chlorophyll and other solutions is so great that there can be no doubt that it is the quality, not the intensity, of the light transmitted through chlorophyll that is effective in producing opening of stomata. In other words, only the wave-lengths most strongly absorbed by chlorophyll are appreciably active in causing opening of stomata in the green leaf.

b. *Effect of light on stomata separate from chlorenchyma:* To avoid the wound response, these experiments were performed on variegated leaves, chiefly of *Pelargonium, Abutilon, Pandanus veitchii,* and a species of grass. The object was to determine whether the guard cells or the chlorenchyma or both absorb the light which causes opening.

KÜMMLER (**12**), using these and other variegated species, found only minimal opening in white parts with medium humidity and normal light and temperature, although in air saturated with moisture he found that light might induce maximal opening in some of the stomata of white areas. VELSEN (**26**), using the porometer method, found much less opening in parts of *Ficus* leaves which lacked green tissue.

In all these plants there is some pigment in the plastids of the guard cells. In *Abutilon* it is distinctly green; in *Pandanus* a very pale green; and in white-edged *Pelargonium,* according to my observation, very pale yellow or greenish yellow and hard to perceive. KÜMMLER describes the guard cells of this form as "chlorophyll free," but there seems to be some pigment present. Whether the pigmentation is distinct or indistinct, it is in each case equal in the green and white leaves or parts of a leaf. There seemed to be no correlation between pigmentation of the guard cells and reactivity to light.

With white or red light, the *predominating influence of green mesophyll* is conspicuous. In turgid leaves on the plant and leaves lying in water when exposed to light which causes the stomata of green parts to become wide open, little effect may be produced on the white parts. The stomata remain closed or open slightly, except that *near the boundary of the green, the stomata on the white areas open well and the influence spreads, as exposure to light continues.* Reduced amount of chlorophyll is accompanied by reduced opening, as was already noted by KÜMMLER. In the pale, but not white, central portion of a banded *Geranium* leaf the stomata were found to be half open when in the green margin they were fully open with circular shape.

Light which has been filtered by a chlorophyll solution has as previously indicated, no more effect on the stomata of white leaves than of green. As the chlorophyll plus xanthophyll absorbs the shorter wave-lengths, and as this end of the spectrum is most chemically and physiologically active, the effect of blue-violet light was studied with aid of a screen. Light from a 150 watt lamp at 6 inches' distance, after passing through a water screen and through a Corning glass filter no. 10 (which allows only blue and violet to pass), has scarcely any effect on stomata either in the pale or white portions of the leaves. Sunlight similarly filtered had a slight effect on both, although how much of this was due to rise of temperature is uncertain. At any rate the effect is small, compared with that of red light on green tissue.

A possibility which has to be considered is that light may stimulate the guard cells directly but does so in full measure only when by its action on the chlorenchyma it has produced a favorable environment. The fact that red light alone produces opening just as well as white points to a pure photosynthetic action rather than to a combination of this and protoplasmic stimulation. Also STALFELT's finding that response varies as light intensity × time of exposure suggests that light supplies the energy and not merely the stimulus. The inference is that in so far as light acts directly on the guard cells, it is through the activity of their chlorophyll. At any rate, it is clear from these experiments that stomata separated from assimilating tissue are only slightly affected by the action of visible light of any wavelength.

The fact that some influence may spread from the green tissue for a certain distance into a white area is in harmony with my theory that reduction of CO_2 concentration in the tissue is a fundamental part of the mechanism of light action; but it does not amount to proof of this mechanism because the result may be due to diffusion of some other substance or the transmission of a physiological stimulus, as in the wounding effect.

The feasibility of the former view is supported more directly by experiments on the effect of varying the concentration of CO_2 and the pH of the guard cells in other ways, with or without exposing the leaves to light.

V. Effects of chemical agents (including CO_2) which change the pH of the guard cells

Preliminary to a study of the rôle of CO_2, inquiry was made as to whether the pH change in the guard cells induced by light is merely accidental or is a part of the mechanism of opening. The answer to this question will depend largely upon whether an artificial variation of pH (especially within the range which is induced by light changes) can produce the full effect of light on the guard cells.

The writer's earlier experiments on this subject have already been referred to. In ammonia solutions and in darkness, starch disappears from the guard cells at a rate which increases with the concentration of alkali up to the limits of toxicity. In the higher concentrations the rate of disappearance is considerably faster than in light. In acid up to a certain concentration, starch is formed in these cells even in the light; but within a narrow range of acidity beyond this and short of a concentration that is rapidly toxic, starch disappears. Prolonged exposure, even to this concentration of acid, results in death of the cells. Accompanying these starch variations reciprocal changes in osmotic value take place with corresponding movements of the guard cells. Under the conditions described, although the starch content may vary from zero to the maximum, the range of movement is small compared with what is found under ordinary conditions in intact leaves. At first sight, therefore, it might seem that the response to these chemical agents is not comparable with the response to light. On the other hand, in sections the wound stimulus and the effect of the aqueous medium tend to prevent opening. In fact, the stomata near a cut surface open to a less extent under the action of light than of ammonia.

Further experiments were therefore carried out, using cut shoots and rooted plants and exposing them to ammonia and acetic acid in vapor form. SAYRE (**20**) has already reported that the stomata of *Rumex patientia* open to 50 per cent. of their maximum in ammonia vapor in darkness and close in an acid atmosphere in light.

In the present experiments the plants were kept in a moist atmosphere under a bell jar, so that the leaves were turgid. The pressure of the reagents in the atmosphere of the bell jar was not measured, but in the case of ammonia, at least, the leaf pigments serve as natural indicators of the concentration of OH-ions in the cells. This is much more significant, because the concentrations of the ammonia or acid vapor outside and inside a leaf do not necessarily vary together. Ammonia strong enough to cause quick color change in leaves with open stomata may have no effect, even after many hours' exposure, on leaves with stomata completely shut.

It was found that under the conditions now described the stomata of *Zebrina* may open almost as widely in ammonia in the dark as under the action of light. As to the intracellular pH at which this occurs, in most experiments the stomata opened only when the purple epidermal cells had changed to green and the guard and subsidiary cells to yellow; that is, at a pH of about 7.4. This condition is usually attended by the formation of a precipitate in the sap of the subsidiary cells, but there is no fall in their osmotic value at this stage (if anything, a slight rise), so that the wide opening is not due to reduction of the pressure of these cells, as might be the case when the ammonia is toxic.

There is evidence, however, that stomata open below pH 7.4. In one case the stomata were found to be wide open with starch reduced almost to nil when the flavone pigment had not become yellow and the anthocyanin had merely changed to a bluish purple shade. Moreover if, as usually happens when the stomata are initially shut, the color change starts from local points of entry of the ammonia and extends as green patches with a blue margin, the stomata in this blue transitional zone, in which there is no yellowing of the guard cells or any other cells, begin to open somewhat rapidly, although the accumulation of ammonia produces further color change before the opening is complete.

It is inferred from these results that if the ammonia concentration could be controlled, full opening would take place when the pH of the guard cells is just short of the point at which the flavone becomes yellow, about pH 7.4.

The indicator tests reported earlier show that in light the pH of the guard cells may rise at any rate to between pH 6 and pH 7, but do not warrant stating whether it passes pH 7 or not.

The conclusion reached is that light causes the guard cells to approach and, in absence of contrary evidence, possibly to reach a pH which in absence of light will induce almost as wide opening as takes place in presence of light. The inference that light acts through changing the pH is rendered more probable by the fact that, as already reported, numerous intracellular changes in the guard cells are the same in natural and artificial opening. Some of these may be accounted for by the change in shape and pressure of the vacuole(s); others by changes in the physical properties (surface tension, viscosity, etc.) of the protoplasm determined by H-ion concentration.

ACTION OF ACETIC ACID VAPOR.—It was found that the action of acid vapor, if not too concentrated, inhibits opening. Opening was sometimes observed in higher concentration but this was not studied, since the relation to carbonic acid seemed to call for more particular attention.

ACTION OF CARBON DIOXIDE.—When the work of DARWIN (**2**), LLOYD (**16**), and LINSBAUER (**15**) made it clear that light acts equally well in absence of CO_2 (one of the raw materials of photosynthesis), and that, according to LINSBAUER, absence of CO_2 is even favorable to opening, including such as may take place in the dark, the old view that the turgor of the guard cells increased through accumulation of the direct products of photosynthesis had to be abandoned. These facts, however, did not upset the general conclusion to which we have been approaching: that photosynthesis is the active factor in stomatal opening, for even in absence of atmospheric CO_2 the photosynthetic process is still carried on in as far as CO_2 is liberated by respiration. Consequently, STAHL (**24**) put forward the view that light acts through removal of CO_2 and darkness through accumulation of the gas as a product of respiration. This conclusion was based especially on LINSBAUER'S experiments, in which he found not only that a decreased amount of CO_2 favors opening but that an increased amount causes shutting both in light and in darkness. According to LINSBAUER, this is true of high partial pressures of CO_2 and of the pure gas; but KOSAROFF (13) found that the reduction of transpiration in high concentrations of CO_2 was the result rather of lack of oxygen than of abundance of CO_2. By mixing oxygen with the CO_2 the depression of transpiration was reduced as the oxygen was increased.

It seems necessary that the relation to CO_2 and oxygen should be further investigated, especially in the light of the following experiments.

Pot plants, cuttings (rooted and unrooted) set in water, and cuttings not in water, were exposed to various concentrations of CO_2 in a dark moist chamber. In the case of the controls, which were kept under similar conditions but in ordinary atmosphere as regards CO_2, there was a certain amount of stomatal opening in the pot plants after many hours but little or none in the cuttings.

In CO_2 of 15 per cent. concentration and upward, the stomata began to open after a period of many hours (12, 24, etc., depending on the concentration), and ultimately became and remained wide open. If initially open, they first closed and then reopened. Moderate wilting, such as occurred in the cuttings which were not in water, did not prevent opening.

Greater opening than in the controls was observed in 10 per cent. CO_2. The rate and degree of opening increase with the concentration of CO_2 until the latter becomes toxic. The occurrence of injury depends greatly on the time of exposure, so that no definite limit of concentration can be named. Even 15 or 20 per cent. causes injury after two or three days, while concentrations over 50 per cent. are endured for several hours. The injury, however, is long subsequent to the opening and is not its cause. The cause is increased turgor pressure (up to the equivalent of $\frac{M}{2} \cdot CaCl_2$) of the guard cells, due to conversion of starch to sugar, presumably as a consequence of the high acidity.

The effect of increased concentrations of CO_2 in light is usually to prevent opening. An atmosphere in which the gas is present in sufficient concentration to cause opening when in darkness may prevent it in light, no doubt because the CO_2 concentration inside the leaf is reduced by photosynthesis.

In some cases the stomata were found wide open in high CO_2. This might be explained by weakness of the light in these experiments, being insufficient to reduce the CO_2 concentration in the leaf to the closing level.

As regards the effect of a *reduced concentration* of CO_2 in absence of light, this is not easy to test, because if the leaf is intact the gas accumulates inside it through respiration, while if the guard cells are exposed by cutting the wound stimulus inhibits opening. It was found, however, that in guard cells exposed to CO_2-free water there is much less accumulation of starch than in those exposed to ordinary water. There is even some disappearance of starch and slight opening if the stomata are shut at the beginning.

The fact that it takes several hours for shut stomata to open under the influence of CO_2, probably on account of the time required for the gas to diffuse into the leaf, and the fact that open stomata close to some extent before they finally open, may account for the difference between LINSBAUER'S results and those now presented.

In brief, these preliminary experiments not only confirm the results of LINSBAUER—that increased concentration of CO_2 in the atmosphere may cause closure in light—but also in every case yield the result that beyond a certain concentration CO_2 causes permanent opening in darkness. This concentration is less than would develop inside the leaf if all the oxygen of a normal atmosphere were replaced by CO_2 through respiration.

These results lend support to a suggestion made in a former paper that stomata regulate against excess as well as against deficiency of CO_2 in the leaf, and that opening after prolonged absence of light may be due to accumulation of the gas. It is not necessary, however, to assume that CO_2 is the only acid product of respiration in the guard cells or other cells which is resynthesized and disappears under the action of light, since it has been demonstrated that in succulent plants organic acids accumulate by night and disappear by day. In fact, it is theoretically not improbable that the semianaerobic condition of a leaf which has been kept in darkness for some time will result in incomplete oxidation of carbohydrate and production of organic acids. Whether other acids are produced or not, the relation between stomatal opening and CO_2 concentration harmonizes well with the relation between opening and acidity of the guard cells already noted. The pH of closure is such as could be induced by lower concentrations of CO_2 and that of acid opening by higher concentrations, provided the cells are weakly buffered. Apparently also, absence of CO_2 without the aid of light produces a pH at which hydrolysis will take place. Whether, in addition to this, light stimulates opening in some other way cannot be said, but at least it can do so only with a sufficiently high pH in the guard cells.

Discussion

A. EFFECT OF LIGHT VS. DARKNESS

Light effects stomatal movement by means of that portion of its spectrum which is absorbed by chlorophyll and principally through its action upon the green tissue of the leaf. Any direct action on the guard cells is either subsidiary to or conditioned by its action through the medium of the chlorenchyma.

Light operates through its photosynthetic activity. The relation of its action to CO_2 concentration, as determined by LINSBAUER and others and confirmed in the present work, defines to some extent the means by which photosynthesis can and cannot act. In the first place, since light is equally or more effective in absence of external CO_2, its full effect can apparently be exerted when photosynthesis is merely sufficient to overbalance the effects of respiration; accumulation of its products is not essential. In the second place, since a moderate increase of CO_2 causes closure even in light, while oxygen is not reduced inside the leaf as it is in darkness but instead is being liberated, it follows that photosynthesis and respiration respectively must act through regulating the pressure of CO_2 rather than of oxygen. This does not exclude the possibility of other modes of action but proves that variation of CO_2 concentration is an effective one.

As regards the relation of stomatal behavior to partial pressure of CO_2, three phases are to be recognized.

(a) *In relatively high concentrations* there is hydrolysis of starch with increase of osmotic value in the guard cells and stomatal opening. This takes place regularly after several hours' exposure to the necessary CO_2 concentration (15 per cent. (?) and over in darkness). The partial opening which sometimes occurs in an ordinary atmosphere after a period of darkness may fall into this class; that is, it may be due to an accumulation of CO_2 in the leaf. This phase of response to CO_2 concentration does not seem to have been recorded previously. The next two are well known.

(b) *In lower concentration* of CO_2 the opposite changes take place in the guard cells together with stomatal closure. Closure in an ordinary atmosphere in darkness and in an atmosphere richer in CO_2 in light both fall into this category when composition of the internal atmosphere of the leaf is the criterion.

(c) *In darkness and absence of* CO_2, at least so far as this condition is attained when epidermal preparations are exposed to water deprived of CO_2, there is a greater tendency to reduction of starch in the guard cells and opening of the stomata than in similar material exposed to water containing CO_2, even at ordinary atmospheric pressure. The opening is only meager, however, but two factors (the wound stimulus due to cutting and the CO_2 produced within the cells, both of which militate against opening) are not present in an intact leaf engaged in active photosynthesis. Under the latter condition, therefore, we should expect better opening. According to LINSBAUER, absence of CO_2 assists opening in light.

While it is clear from the present results that reduction of CO_2 pressure through photosynthesis is an essential part of the action of light, the complete adequacy of this condition to produce full stomatal opening in absence of light is difficult to test and has not been proved by these experiments.

The same three phases of stomatal response as previously noted also appear in relation to the H-ion concentration of the guard cells as governed by other penetrating acids than CO_2 and by penetrating alkali. Acetic acid in relatively high concentration, namely, just short of being rapidly toxic, causes opening; in weaker concentration it causes closure and ammonia again causes opening. This relation was described in an earlier paper. It is now shown that under suitable conditions this regulation of carbohydrate balance, turgidity of the guard cells, etc., by artificially changing the pH of these cells, is as ample and complete as in normal movement. It is independent of and overcomes the action of light or darkness, as well as of variations in leaf turgor.

Vital staining tests with indicators point to the conclusion that light and darkness themselves induce wide fluctuations in the pH of the guard cells, extending from about pH 5 after a period of darkness to between pH 6 and pH 7, or perhaps higher, after a period of good illumination. The experiments do not show whether or not it reaches a point (probably about pH 7.3) at which hydrolysis of starch and opening of the stomata can take place without the aid of light. The possibility remains, therefore, that some action on the part of light in addition to changing the pH is involved when complete opening takes place.

The acid range of pH and therefore the behavior in darkness can be accounted for by production of CO_2 alone if the guard cells are not too well buffered; although, of course, other acid products of respiration may assist in developing a low pH.

B. EFFECT OF LEAF TURGOR

When closure is induced by wilting of the leaf, the guard cells exhibit a *low pH* and other characteristics associated with closure in darkness, even though the leaves are exposed to bright sunlight. Considerable wilting does not prevent opening (accomplished by the usual mechanism) in leaves exposed to amomnia gas or to the higher pressure of CO_2. This seems to prove that within limits, scarcity of available water does not prevent hydrolysis of starch in the guard cells, provided their pH is right for the process; and that wilting, like darkness, acts by creating an H-ion condition conducive to closure. It may do this by inhibiting photosynthesis. The effects of leaf turgor both in light and darkness are dealt with more fully in the paper which follows.

C. EFFECT OF TEMPERATURE

Low temperature tends to produce closure, and high temperature opening of stomata, whether in darkness or in light. Here again, to judge by vital staining, the movement is correlated with the same pH change as under the influence of the other factors.

In a fuller study of the temperature relation to be described in a subsequent paper, it will be demonstrated that the turgor of the guard cells is regulated by the same carbohydrate transformations as in these cases. The pH difference due to temperature cannot depend on photosynthesis, because it occurs in darkness.

D. Effect of wounding

Injury to cells, *e.g.*, by cutting, causes stomata in the immediate vicinity of the wound to close. In this case the loss of turgor in the guard cells is unaccompanied by increase of starch and is probably due to escape of solute; that is, to increased "permeability."

Summary

1. All major stomatal movements in the plants studied, whether due to change of illumination, of leaf turgor, or of temperature, are accompanied by reversible transformation of carbohydrate and also by pH change in the guard cells.

2. The carbohydrate balance depends strictly on the pH.

3. The origin of the pH change, when governed by the light factor at least, depends mainly on the relative rate of photosynthesis and respiration respectively in the leaf tissue in general and demonstrably on the CO_2 pressure in the environment of the guard cells.

4. Absence of CO_2 favors, if it does not of itself induce, stomatal opening.

5. A certain concentration of CO_2 causes closure; a higher concentration, opening.

6. Any response of the guard cells to the direct action of light is small or else conditioned by the environmental state.

7. An entirely different mechanism, namely, increase of permeability, seems to be involved in the stomatal closure which takes place in the vicinity of a wound.

McGill University,
Montreal, Canada.

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COMPARISON OF THE EFFECTS OF TEMPERATURE ON THE RADIAL AND LONGITUDINAL ELECTRIC POLARITIES IN WOOD AND CORTEX OF THE DOUGLAS FIR

E. J. LUND

(WITH FIVE FIGURES)

Introduction

In previous papers it has been shown, chiefly by indirect methods, that all the present known facts about the distribution of correlation potentials in the Douglas fir can be explained by the existence of two oppositely oriented radial E.M.F.s, one of which is located in the wood and the other in the cortex. The radial E.M.F. in the wood increases toward the base while that in the cortex increases toward the apex. The direct demonstration of the separate existence of these two E.M.F.s is not a simple matter, because mechanical removal of the cortex always involves change in its inherent E.M.F.

Since it has been shown that the external polarity of the apex can be increased, decreased, inverted, or made equal to zero by an appropriate change in temperature, it becomes of importance to know how a temperature change affects the two different sets of E.M.F.s in the wood and cortex. The following experiments which bear upon these questions yield further confirmatory evidence for the existence of the two oppositely oriented E.M.F.s. They also point to the conclusion that when the temperature of the tree is changed, the pattern of the internal correlation potentials is changed. This result appears to depend fundamentally upon the general fact that *equal change in temperature changes the E.M.F. unequally in different loci of the axis in polar tissues.* An explanation of this unequal effect has already been presented (LUND **1, 2**).

Procedure

Since nothing is known about the effects of temperature on the internal electric phenomena associated with polarity, the experiments described in the present paper are in a sense exploratory. The plan was to follow nearly simultaneously the changes in the magnitude and direction of the following E.M.F.s in the same stem segment, with the same change in temperature.

1. Longitudinal E.M.F of intact stem.
2. Longitudinal E.M.F of wood axis without cortex.
3. Radial E.M.F. of intact stem.
4. Radial E.M.F. of wood axis without cortex.
5. Diagonal E.M.F. of wood axis without cortex.

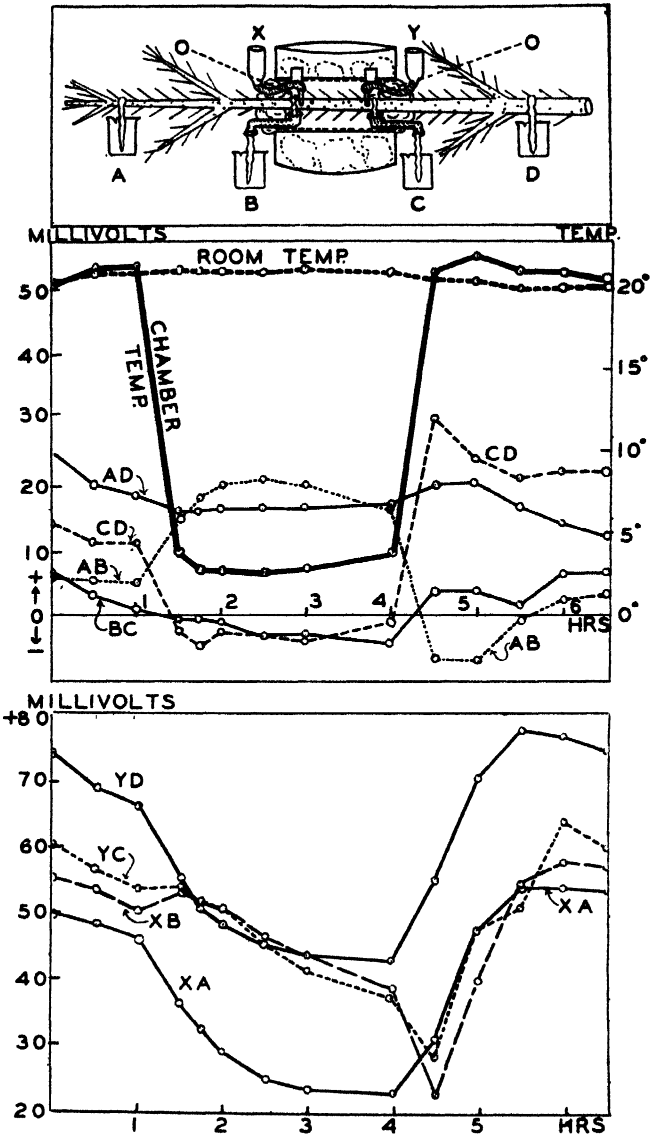

FIG. 1. The diagram at the top shows the arrangement of the apparatus. The cups *X* and *Y* are filled with tap water and connect by rubber tubes and glass cannula to the center of the wood at *O*. The E.M.F. between contacts *X* and *B*, and *Y* and *C* is radial. The third internode was used in the experiment. The distances between the contacts *A* and *B*, *B* and *C*, and *C* and *D* were respectively 22, 12.5, and 19 cm. The lower sets of graphs are separated from those above to facilitate comparisons. Both sets of curves are drawn to the same scale.

6. Diagonal E.M.F. of intact stem.
7. E.M.F.s in dead and living parts of same stem.

For the sake of comparison, the results from four types of experimental procedure will be given. A diagram of the apparatus and contacts used in each of these procedures is given at the top of each of the figures 1, 2, 3, and 5.

Before the results given by the curves are considered, a comparison should be made of the four different experimental arrangements. Note that four contacts labeled *A, B, C,* and *D* in the figures are made in a similar manner in each of the four types of experiments. Two of these electrode contacts (*A* and *D*) were outside the chamber and remained at room temperature throughout the experiments; the other two external contacts were placed inside the chamber, and together with the stem segment were subjected to the change in temperature.

For measurement of the radial and diagonal E.M.F.s two more electrode contacts (*X* and *Y*) were made at the points indicated by inserting a glass tube 2 mm. in diameter to the bottom of a hole drilled exactly to the center of the wood axis. The glass tubes were connected by rubber tubes to cups on the outside as shown in figures 1, 3, and 5. In this way the electrode connection with tap water was made at the center of the wood axis. The duration of the experiments varied from four to seven hours, and as usual all measurements were made with the potentiometer. The first two experiments, given in figures 1 and 2, were made on the third internode from each of the tops of the main stems of two different trees. The second and third experiments, given in figures 3 and 4, were similarly made on the second internode from each of two different trees. The results of the experiments given in the figures are typical of other experiments which will therefore not be reported.

The significance of the results shown in the curves can be appreciated only when a careful comparison is made of the properly selected curves in the different figures.

LONGITUDINAL E.M.F.S IN WOOD AND CORTEX

1. LONGITUDINAL E.M.F. ON WOOD AXIS IS NOT AFFECTED BY CHANGE IN TEMPERATURE.[1]—This fact is shown in part in curve *BC* of figure 2, which should be compared with curve *BC* in figure 1. Cooling one contact placed on the wood axis does not affect the E.M.F. between it and another contact placed either on the wood axis or on the cortex of another region kept at room temperature. Evidence for this statement is found by comparing curve *AB* of figure 2 with curve *AB* of figure 1, and is also shown by curve

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[1] By this statement is meant that within the limits of accuracy in procedure in the present experiments the curves do not show any clearly evident effect.

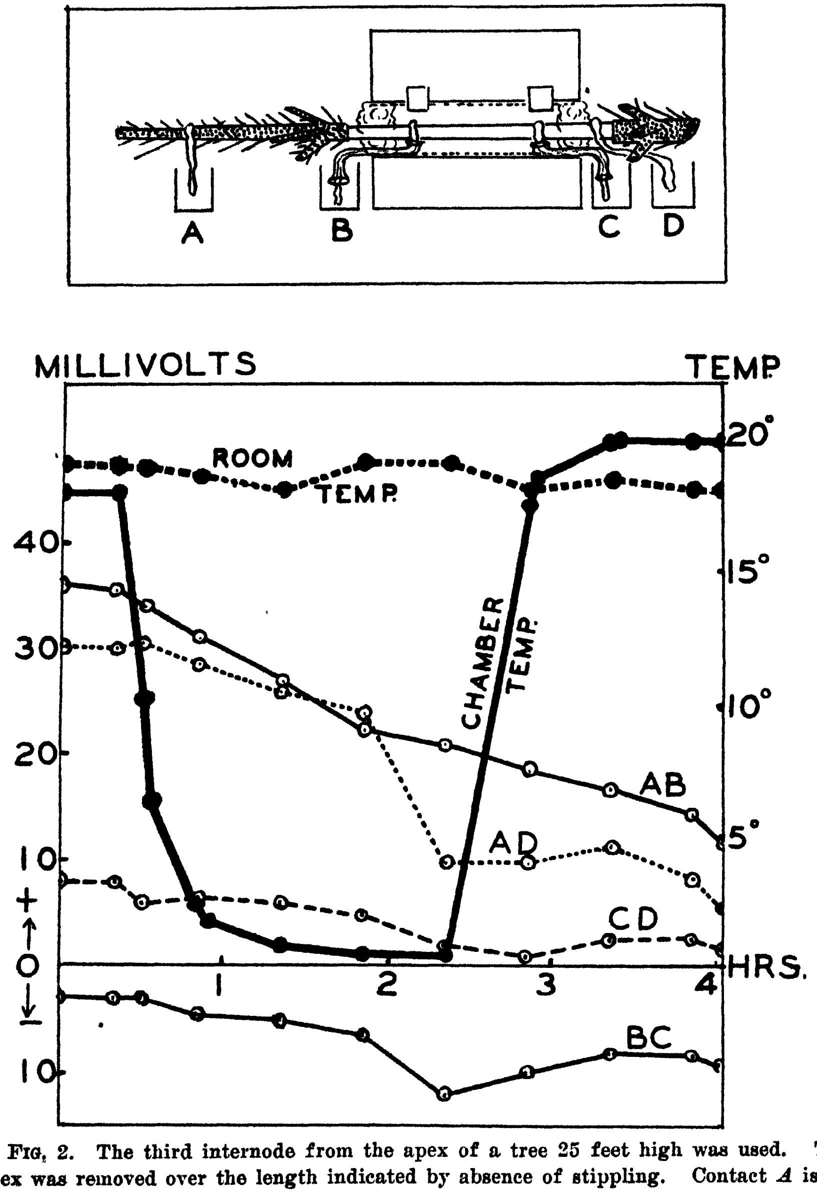

FIG. 2. The third internode from the apex of a tree 25 feet high was used. The cortex was removed over the length indicated by absence of stippling. Contact *A* is on the outside of the cortex; contacts *B*, *C*, and *D* are on the wood axis. Contacts *B* and *C* are subjected to change in temperature; contacts *A* and *D* remain at room temperature. The distances between *A* and *B*, *B* and *C*, and *C* and *D* were respectively 22, 13, and 8.5 cm. The total length of exposed wood axis is 34.5 cm.

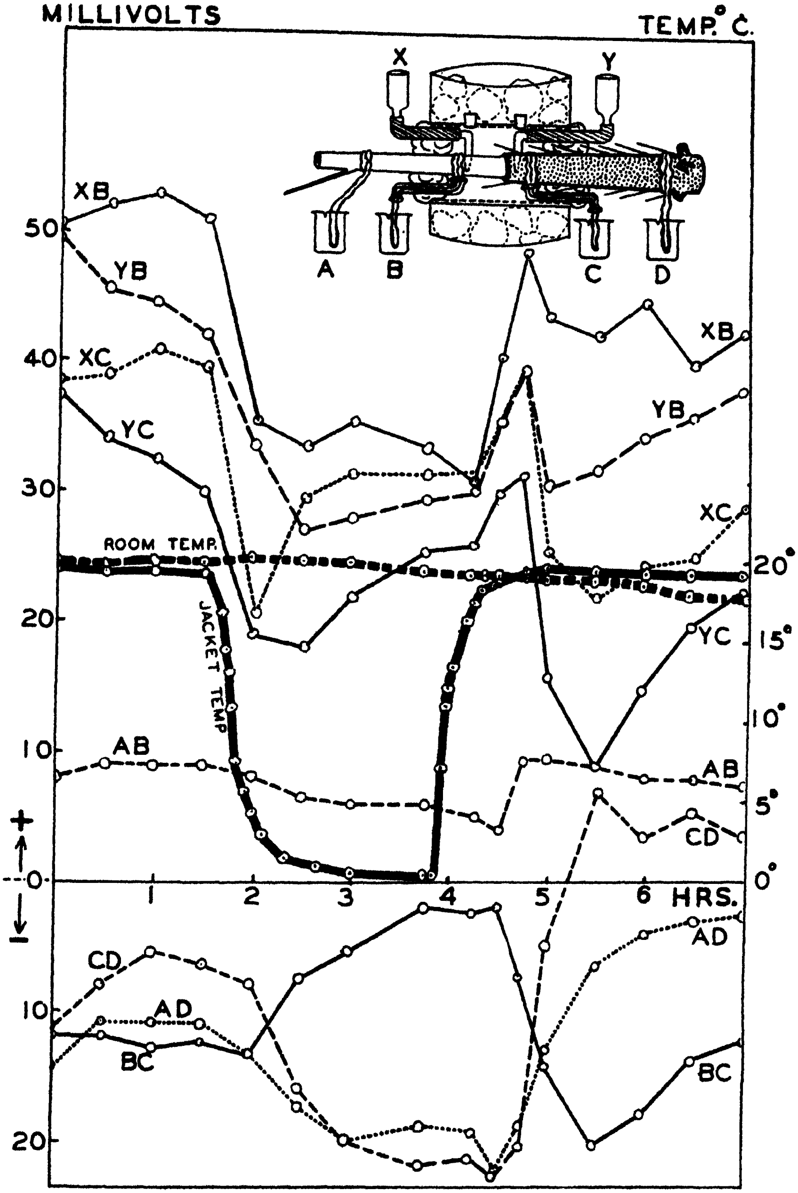

FIG. 3. The second internode from the main stem of a tree was used. The cortex was removed from the unstippled portion of the internode shown in the diagram. Distances between electrodes *A* and *B*, *B* and *C*, and *C* and *D* were respectively 14, 13, and 17 cm.

CD of figure 2. In this latter case both contacts are on the wood axis but one is cooled and the other is not cooled. This curve should be compared with curve *CD* of figure 1.

Again compare curve *AB* in figure 3 with curve *CD* in figures 3 and 1. The sudden bend in curve *AB* of figure 3 is unexplainable as an effect of temperature, and may have been due to an accidental mechanical displacement of the contact.

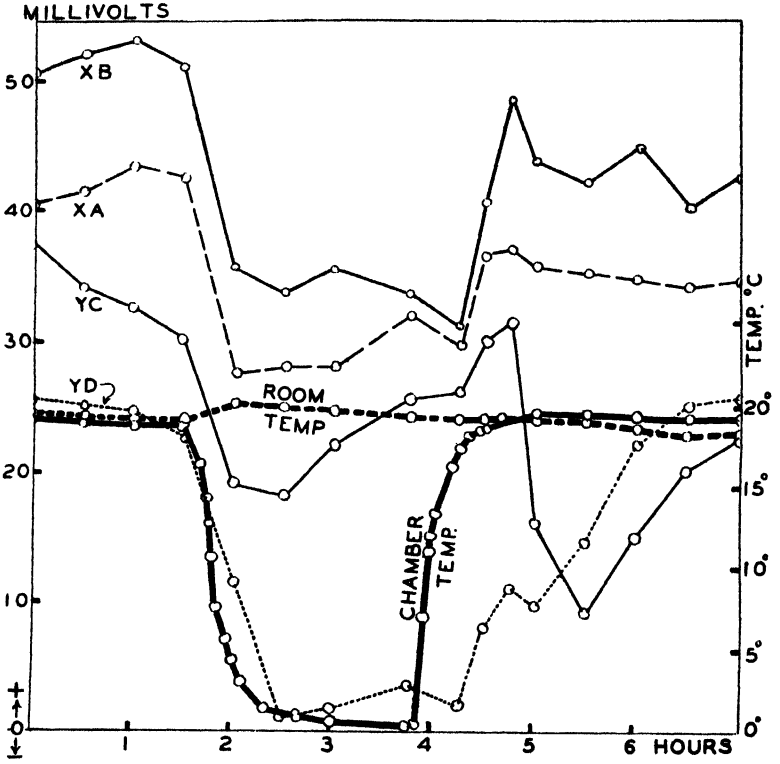

FIG. 4. The curves show the E.M.F.s between the correspondingly labeled electrode contacts in the diagram of figure 3. They are shown separately to facilitate comparison of the effects of temperature. Note that $XB > YC$ and $XA > YD$. The form of curve *YC* is of special significance.

2. LONGITUDINAL E.M.F. BETWEEN TWO CONTACTS ONE OF WHICH IS ON WOOD AND THE OTHER ON THE CORTEX IS CHANGED BY EQUAL CHANGE IN TEMPERATURE OF CONTACTS AND REGION WHICH THEY INCLUDE.—This fact is shown by comparing curve *BC* of figure 3 with *BC* of figure 2. A comparison should also be made between the magnitudes of the effects of temperature in curve *BC* of figure 3 and that in curve *BC* in figure 1. The ap-

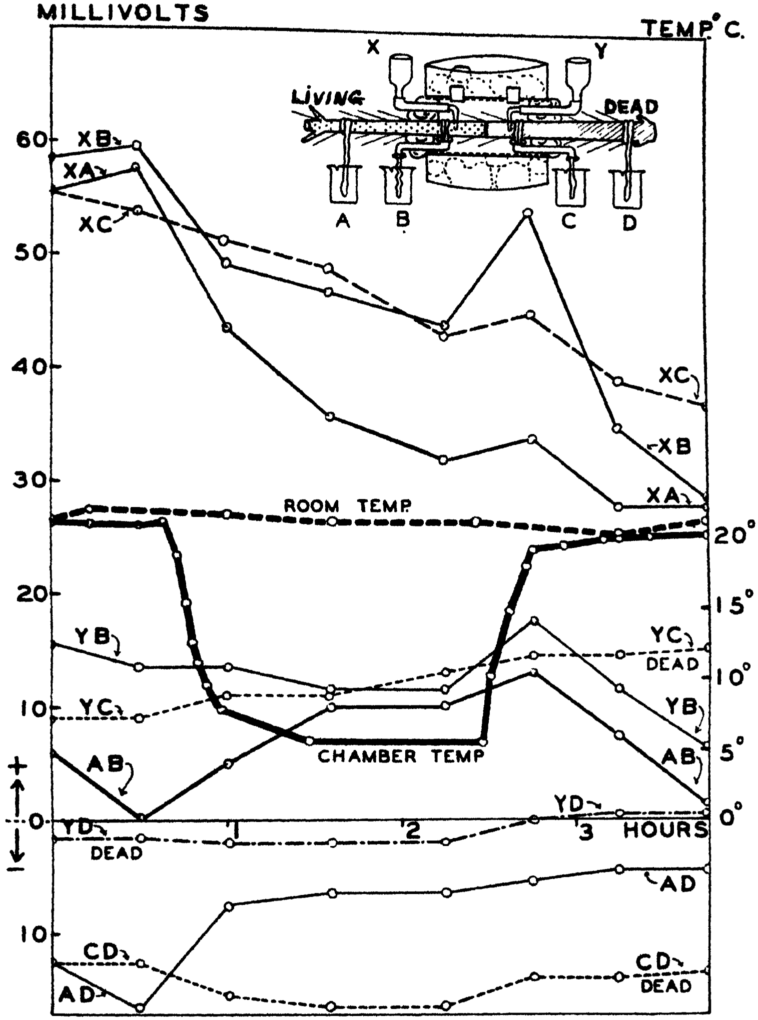

FIG. 5. The curves show the difference between the effects on the E.M.F.s caused by the same change in temperature in a dead and living section of the stem. The second internode of a main stem was used. The basal half of the internode was immersed in boiling water during 4 minutes and then cooled to room temperature, while the apical half was kept cool by wrapping in a cool wet cloth. The distances between the contacts *A* and *B*, *B* and *C*, and *C* and *D* were respectively 15, 14, and 14 cm. The internode was allowed to come to room temperature before the experiment was begun. The E.M.F.s between the contacts *Y* and *C*, *Y* and *D*, and *C* and *D* are only slightly affected by a temperature change which affects markedly the corresponding contacts in the living part of the stem.

parent conclusion from such experiments is that temperature affects the E.M.F. by affecting the cortex. Note that curves *CD* and *AB* of figure 1 and curve *CD* of figure 3 confirm fully all of the corresponding observations presented in the previous paper on the effect of temperature on the E.M.F. in the apex (LUND **2**).

3. WHEN ONE CONTACT IS PLACED ON THE WOOD AND THE OTHER ON THE CORTEX, BOTH CONTACTS BEING KEPT AT SAME TEMPERATURE, THEN WHEN THE REGION OF THE JUNCTION BETWEEN EXPOSED WOOD AND NORMAL STEM IS CHANGED IN TEMPERATURE, THE E.M.F. IS CHANGED.—Curve *AD* in figure 3 demonstrates this fact. This curve should be compared with curves *AD* in figure 2 and *AD* in figure 1. It should also be compared with curve *AF* in figure 2 of the writer's previous paper (LUND **2**). The remarkable fact should be noted that curve *BC,* figure 3, changes in an opposite manner to *AD,* figure 3, when the temperature is lowered around the contacts *B* and *C* and their included segment, but not around the contacts *A* and *D.*

Finally, the effects of temperature as shown in curve *AD,* figure 3, prove conclusively that the E.M.F. measured between contacts *A* and *D* does not all originate at the contacts, and that all the change in E.M.F. does originate in the region of the stem which is cooled. This is a simple but convincing demonstration of the existence of summation of E.M.F.s.

EFFECT OF TEMPERATURE ON RADIAL POTENTIALS IN WOOD AND WOOD-CORTEX SYSTEM

The radial E.M.F. of the wood and the longitudinal E.M.F.s of the intact stem are similarly affected by a change in temperature. This fact is shown for example by the curve *XB* in figure 3. From this it may be concluded that the absence of an effect on the longitudinal E.M.F. of the wood is not due to an absence of effect on the radial E.M.F.

That a lowering of the temperature decreases the radial E.M.F. of the bipolar wood-cortex combination is evident from curve *YC,* figure 3, and curves *XB* and *YC,* figure 1. These curves exhibit interesting characteristics of form which are probably due to the rate of cooling and the opposite orientation of electric polarities in the wood-cambium-cortex system. However, since the present experiments are not intended to show more than some of the more important general characteristics of the effects of temperature, a fuller analysis of the precise course of the effects of change of temperature upon any one type of measurement must be omitted for the present.

EFFECT OF TEMPERATURE ON DIAGONAL E.M.F.S

Curves *XA* and *YD* in figure 1 show a closely parallel effect. It should be observed that these measurements were on the intact stem. Curves *XA* and *YD* in figure 4 should be compared. Note that the E.M.F. of *XA* is greater than the E.M.F. of *YD,* as it should be if the inherent E.M.F. in the wood and the cortex are oppositely oriented. Note also that this relation in magnitude is not true when curves *XA* and *YD* of figure 1 are compared. This again fulfils expectations, as mentioned later.

All experiments show that lowering of temperature lowers the diagonal E.M.F.s.

INHERENT E.M.F. IN CORTEX

The following facts derived from a comparison of the curves show that an inherent E.M.F. exists in the cortex. Curve *AB,* figure 3, and curves *AB* and *CD,* figure 2, show that the longitudinal potential at and in the region of the contact *B* on the wood is not affected by a change in temperature. On the other hand, curves *CD* in figures 1 and 3, and curve *AB* in figure 1 show that changing the temperature on a contact on the cortex and its adjacent region changes the E.M.F. between the contacts. Again, curve *BC* of figure 3 shows that change in temperature affects the E.M.F. between the contacts *B* and *C*, one of which is on the wood and the other on the cortex. But curve *BC* of figure 2, where both contacts are on the corresponding regions of the wood, shows no such effect.

Finally, curve *AD* in figure 3 shows that temperature affects the E.M.F. between these two contacts even though they themselves are not cooled, while curve *AD* in figure 2 shows no such effect of cooling on its E.M.F. It follows from these facts that the cortex in the region *C* in figure 3 is the seat of an inherent E.M.F. whose magnitude is markedly affected by a change in temperature. These facts, when taken in conjunction with certain facts presented in previous papers (**2**), appear to constitute a definite proof that the cortex possesses an inherent E.M.F.

The second question of special interest is, what is the orientation of the E.M.F. in the cortex?

Curve *BC* in figure 3 shows that contact *B* on the wood is electronegative in the external circuit to contact *C* on the cortex. In the same way curve *AB* in figure 2 shows that *B* is electronegative to *A*.[2] Now, since the E.M.F. at the contact *B* and its region on the wood is *not* affected by a change in temperature, as shown by curves *AB* and *CD* in figure 2, while the E.M.F. between contacts *B* and *C* in curve *BC* of figure 3 is changed by a change in temperature, then the E.M.F. between *B* and *C* in figure 3 must lie in the cortex as just shown. Since the surface in contact with *C* is electropositive to *B,* then this must mean that the E.M.F. of the cortex is oriented in such a way that the outside of the cortex is electropositive to the inside of the cortex in the external circuit.

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[2] The curve *AB,* figure 2, is shown *above* instead of below the base line in order to comply with the convention adopted in labeling polarities as given earlier in the paper.

Furthermore it is shown in figure 4 that the E.M.F.s of *XB* and *XA* are respectively greater than the E.M.F.s of *YC* and *YD*. This relation is never observed in the intact stem, such as that in figure 1 where $YD > XA$ and $YC > XB$. These differences can be explained if we assume that an E.M.F. exists in the cortex, this E.M.F. having an opposite orientation to that in the wood. These facts again indicate the same conclusion as that arrived at in a previous paper (LUND **4**) by different methods of experimentation.

The results suggest that the longitudinal E.M.F.s observed when the leads are attached to the intact outer surface of the cortex are probably mostly derived from the inherent E.M.F. in the cortex. This conclusion seems to be strengthened by the demonstrated fact that the external polarity in the apical region is much greater than that in the more basal regions, in spite of the fact that the thickness of the wood and therefore the radial E.M.F. in the wood diminishes to small value in the apex.

Finally it may be recalled, as shown in a previous paper (**4**), that the diagonal basal-apical (inside to outside) E.M.F. in the apex is oriented oppositely to that in the lower parts of the stem.

All the diverse facts seem to leave no doubt that the inherent E.M.F. in the cortex is oriented oppositely to that in the wood. This conclusion agrees of course with the morphological and functional bipolar character of the cambium.

General conclusions

With the possible exception of the external longitudinal E.M.F.s on the isolated wood axis, all the observed E.M.F.s are markedly affected by a change in temperature. In all of these cases it appears that when the temperature of both electrode contacts and the whole region of the stem involved in the measurement of the E.M.F. is lowered, the observed E.M.F. decreases. In other words, all the observed results appear to be due to the simple but apparently fundamental fact that *equal* change in temperature causes an unequal change in the E.M.F.s along the polar axes, whether these be longitudinal, radial, or diagonal resultants of the elementary polarity potentials of the living cells. This principle reminds us of the similar unequal effects on the velocity of oxidation in apical and basal ends of the stem of *Obelia*, produced by equal change in oxygen concentration.

The present experiments indicate that when the temperature of the tree system is changed uniformly throughout, or when one part of it is subjected to a change in temperature which is different from the temperature maintained in other parts, the *relative magnitude* and even the *directions* of flow of the electric correlation currents must become changed in a definitely characteristic manner. The possible consequences associated with such a change in the pattern of the electric correlation mechanism appear now of paramount interest. Experiments will deal with these problems in the papers which are to follow.

COMPARISON OF EFFECT OF CHANGE IN TEMPERATURE UPON THE LIVING AND DEAD STEM

Only one experiment is given to show the typical differences in behavior of the E.M.F.s in a dead and in a living stem. The results are given in figure 5. In this experiment the readings of E.M.F. were made at longer time intervals and therefore the curves do not bring out the differences as clearly as they otherwise would have done.

The basal half of the second internode of the main stem of a tree was killed in boiling water, cooled to room temperature, and set up in the apparatus as shown in the diagram at the top of figure 5. After allowing the preparation to rest for one-half hour, the readings of E.M.F.s were taken as shown in the curves.

The first fact to notice is that E.M.F.s exist in the dead half of the stem just as we might expect to find in a telephone pole, chimney, or between two sides of many other objects. An intelligent appreciation of the facts, however, leads to the conclusion that the behavior of the E.M.F.s in the dead part of the stem when subjected to change in temperature is radically different from the corresponding ones in the living stem. This fact is shown by a comparison of the effects of temperature on the radial potentials, curve *YC* which is dead and curve *XB* which is living. Similarly, curve *XA* (living) and curve *YD* (dead) should be compared. Curve *CD* (dead) is to be compared to curve *AB* (living). The general fact observed in the curves is that whenever both contacts lie in and include only dead stem between them, the E.M.F. is not affected by temperature, except to a small extent as would be expected if they are concentration potentials of various types in a non-living system. This is shown by curves *YD, CD*, and *YC* in figure 5.

Summary

1. The longitudinal and radial E.M.F.s in the intact stem below the apex of the Douglas fir are both greatly decreased by lowering the temperature and increased by raising it. The radial E.M.F. of the isolated wood axis is affected in a similar manner. Dead stems often show E.M.F.s but these are not affected by temperature like those in the living stem.

2. By changing nearly simultaneously the temperature of the wood and cortex, it is possible to show that the cortex is the seat of an electric polarity oriented in the opposite direction to that in the wood. The radial E.M.F.

in the cortex appears to be equally or more sensitive than that in the wood to the same change in temperature.

3. The same change in temperature causes a greater change in the E.M.F. of the cortex in apical regions than in the cortex of relatively more basal regions.

4. The phenomenon of "rebound" of the E.M.F. after a period of exposure to low temperature is less pronounced in lower regions of the stem than in the apex. Additional evidence shows that summation of E.M.F.s occurs.

5. All of the observed effects of temperature on the various polarities in the living tree appear to be due to the simple but apparently fundamental fact that equal change in temperature causes an unequal change in the E.M.F. along the polar axis under consideration. In all cases the region having the highest relatively electropositive condition with respect to the external circuit is changed by the greatest amount.

6. Temperature acts as one of the primary environmental factors which can control the magnitude and even the direction of the internal electric polarities in the tree system. Change in temperature can and does change the pattern of the internal electric correlation currents in the Douglas fir.

UNIVERSITY OF TEXAS,
AUSTIN, TEXAS.

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DETERMINATION OF THE INORGANIC NITROGEN IN THE CORN PLANT BY THE EXPRESSED SAP METHOD

R. W. GERDEL

Introduction

In the course of some studies on the chemistry and physiology of the corn plant, it was found by SAYRE and MORRIS (1) that the total sugar content could be determined from the analysis of an aliquot of sap expressed from the ground tissue. Since the determination of the inorganic nitrogen content of corn plants grown on plots to which nitrogen fertilizer was applied in several forms and amounts, and by several methods of application, was a part of the problem under investigation, an opportunity was afforded for determining the application of the expressed sap method to the determination of the total inorganic nitrogen.

Material and methods

The sap or juice of the plant was obtained by subjecting 100 gm. of fresh material, previously ground through the medium knives of a hand meat grinder, to a pressure of 5000 pounds per square inch by means of a small laboratory hydraulic press. The green and dry weights of the press cake and the specific gravity of the sap were recorded. The percentage of total solids in the sap was determined by the refractometer, and the weight of sap was calculated by subtracting the green weight of the press cake from the weight of the original sample, in this case 100 gm.

The aspiration method of SESSIONS and SHIVE (4) appeared to be best adapted to the determination of the inorganic nitrogen content of the expressed sap. A slight modification of the apparatus was made, 300-cc. Kjeldahl flasks being substituted for the Florence flasks used in the original procedure. The rounded bottom of the Kjeldahl flask permitted the aspiration tube to come in closer contact with the Devarda's alloy, and consequently more of the alloy was held in suspension. The long neck of the Kjeldahl flask also provided more room for the expansion of the foam which occurred with sap from leaf tissue. This foaming was not entirely suppressed by the use of either paraffin oil or caprylic alcohol. The ammonia evolved through aspiration was determined by the boric acid method of SCALES and HARRISON (3). The large absorption tubes as used by SESSIONS and SHIVE each contained 50 cc. of a 4 per cent. boric acid solution and 0.3 cc. of brom-phenol-blue indicator.[1] The ammonia was titrated directly with

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[1] Preparation of brom-phenol-blue indicator: 0.4 gm. brom-phenol-blue heated with 12 cc. N/20 NaOH until dissolved and diluted to 500 cc. with distilled water.

N/20 sulphuric acid. No loss of ammonia could be detected through the use of the 4 per cent. boric acid solution under the slightly reduced pressure occurring in this apparatus. Since the concentration of the boric acid solution was only approximate, a considerable saving in labor resulted, due to the necessity of preparing only one standard reagent. Titration by reflected light from a 100-watt daylight bulb produced a very sharp end-point with brom-phenol-blue indicator.

The formulas for calculation of results are developed from the basic formula used by SAYRE and MORRIS (**2**). Using figures obtained in this study (table I), this basic formula has been adapted to the determination of total inorganic nitrogen as follows:

TABLE I

METHOD OF CALCULATING RESULTS

WEIGHT FIELD SAMPLE			WEIGHT PRESS CAKE FROM 100 GM. LAB. SAMPLE		SAP FROM STALK TISSUE			
LEAVES	STALK	TOTAL	GREEN	DRY	WEIGHT	SP. GR.	TOTAL SOLIDS	N IN 10 CC.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		*per cent.*	*gm.*
2135	2162	4297	21.7	11.7	78.3	1.046	11.85	0.00196

$$\text{Per cent. } H_2O = \text{weight lab. sample} - (\text{per cent. total solids} \times \text{weight sap} + \text{dry weight of press cake})$$

$$79 \text{ per cent.} = 100 - (0.1185 \times 78.3 + 11.7)$$

$$\text{Total } H_2O \text{ in stalk} = \text{per cent. } H_2O \times \text{stalk weight}$$

$$1709 \text{ gm.} = 79 \text{ per cent.} \times 2162$$

$$H_2O \text{ content of expressed sap aliquot} = (\text{vol. of aliquot} \times \text{sp. gr.}) - (\text{vol. of aliquot} \times \text{per cent. total solids})$$

$$9.27 = (10 \times 1.046) - (10 \times 0.1185)$$

$$\text{Total inorganic N in stalk} = \frac{\text{gm. N in aliquot}}{H_2O \text{ content of aliquot}} \times \text{total } H_2O$$

$$0.3615 \text{ gm.} = \frac{0.00196}{9.27} \times 1709.$$

Experimentation

AMOUNT OF REAGENTS REQUIRED

In the preliminary stages of this study, considerable variations in the nitrate nitrogen content of duplicate samples were found. This excessive variation between duplicates was overcome by the use of an additional 2.5 gm. of Devarda's alloy and 5 cc. of 6 per cent. NaOH, and by a longer aspiration period, as shown in tables II and III.

TABLE II

AMOUNT OF REAGENTS REQUIRED FOR COMPLETE ASPIRATION OF NITRATE NITROGEN IN 10 CC. CORN SAP BY SESSIONS AND SHIVE METHOD

SAMPLE	NITRATE NITROGEN RECOVERED						TOTALS AVERAGED
	DUPLICATE 1			DUPLICATE 2			
	1ST* RUN	2D* RUN	TOTAL	1ST* RUN	2D* RUN	TOTAL	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	5.93	1.96	7.89	7.28	1.54	8.82	8.355
2	6.96	2.52	9.48	7.77	1.89	9.66	9.570
5	4.76	2.52	7.28	5.60	1.96	7.56	7.420
6	4.48	2.38	6.86	5.60	1.88	7.48	7.170
8	6.23	3.08	9.31	8.19	1.88	10.07	9.690

* 2.5 gm. Devarda's alloy and 5 cc. of 6% NaOH added and aspirated 14 hours for first run; after titration another 2.5 gm. of Devarda's alloy and 5 cc. of 6% NaOH were added and aspirated for another 8 hours.

Note: each 0.1 cc. of N/20 acid is equivalent to 0.07 mg. NO_3 nitrogen.

TABLE III

TIME REQUIRED FOR COMPLETE ASPIRATION OF NITRATE NITROGEN IN 10 CC. CORN SAP BY SESSIONS AND SHIVE METHOD*

SAMPLE	NITRATE NITROGEN				
	DUPLICATE 1			DUPLICATE 2	AVERAGE
	1ST 8 HOURS	2D 8 HOURS	TOTAL	16 HOURS	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
.	2.10	0.63	2.73	2.59	2.660
	3.92	1.40	5.32	5.53	5.425
	2.87	1.12	3.99	3.92	3.955
.	2.73	1.12	3.85	4.20	4.025
.	4.83	1.89	6.72	6.93	6.825
...	2.52	1.26	3.78	3.78	3.780

* 5.0 gm. Devarda's alloy and 10 cc. 6% NaOH added.

Note: each 0.1 cc. of N/20 acid is equivalent to 0.07 mg. NO_3 N.

Apparently, close agreement between duplicate samples may be obtained by the use of 5 gm. of 100 mesh Devarda's alloy and 10 cc. of 6 per cent. NaOH, while an aspiration period of approximately 16 hours appears necessary to remove completely all the inorganic nitrogen in the expressed sap sample.

RECOVERY OF ADDED NITRATES

Since in no case, throughout the 1930 season, was the ammonia form of nitrogen found in the corn sap in more than a doubtful trace, studies on the accuracy of the combined "expressed sap" (SESSIONS and SHIVE method) were made on inorganic nitrate salts. The efficiency of the method in the recovery of the nitrate nitrogen added as sodium nitrate to both sap and distilled water is shown in table IV.

TABLE IV

RECOVERY OF NITRATE NITROGEN FROM EXPRESSED SAP BY SESSIONS AND SHIVE METHOD

COMPOSITION OF SOLUTION	TOTAL NO_3 N CONCENTRATION			TOTAL NO_3 N RECOVERED		
	DET. 1	DET. 2	AVERAGE	DET.* 1	DET.* 2	AVERAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
10 cc. dist. H_2O plus 7.0 gm. N as $NaNO_3$	7.00	7.00	7.000	6.79	6.79	6.79
10 cc. expressed sap				4.62	4.55	4.59
					Total	11.38
10 cc. expressed sap plus 7.0 mg. N as $NaNO_3$				10.99	11.55	11.27

* Each determination is the average of duplicates.

The sum of the averages for the separate determinations of the nitrate nitrogen content of an aqueous solution of sodium nitrate and a 10-cc. aliquot of sap is in close agreement with the nitrate nitrogen content of a 10-cc. aliquot of the same sap to which an equivalent amount of nitrogen as sodium nitrate was added.

ACCURACY OF EXPRESSED SAP METHOD

The accuracy of the expressed sap method for the determination of any constituent of the corn plant, which is to be expressed as a part of the whole plant, is dependent upon the proportional quantity of that constituent which will be found in the sap. If the total sap removable from 100 gm. of green tissue is separated into successive aliquots as the pressure on the sample is increased, and if a like amount of the constituent being studied is found in each successive portion of the sap, then it may safely be concluded that the moisture not removable from the tissue at a pressure of 5000 pounds per square inch contains an amount of this constituent proportional to the non-expressable moisture content of the tissue. That such is the case where the nitrate nitrogen content of the corn plant is being studied is shown by the data in table V.

TABLE V

Nitrate nitrogen in successive portions of corn sap

Part of plant	1	2	3	4	5	6	Average
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Leaf tissue, six 10-cc. portions	1.155	1.225	1.155	1.045	1.085	1.085	1.102
Stem tissue, five 15-cc. portions	2.345	2.310	2.275	2.310	2.310		2.310

Note: leaf tissue sample of July 17, stem tissue sample of July 31; all results are averages of duplicate determinations.

There is a close agreement between successive portions of sap obtained from both leaf and stem tissue at increasing amounts of pressure, indicating that the nitrate nitrogen is probably in aqueous solution in the plant and the amount obtained in the sap is proportional to any fraction of the total moisture expressed. Further evidence in support of this hypothesis is shown by the close agreement between the nitrate nitrogen content of the alcoholic extract of an aliquot of ground tissue and the calculated nitrate nitrogen content determined by the expressed sap method on duplicate aliquots of tissue (table VI).

TABLE VI

Comparison of expressed sap and alcoholic extraction methods

Method	Nitrate nitrogen as percentage green weight					
	1 A	1 D	14 A	14 D	17 A	17 D
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Expressed sap	0.0268	0.0261	0.0349	0.0531	0.0251	0.0298
Hot alcoholic extraction	0.0266	0.0259	0.0350	0.0517	0.0252	0.0281

Preparation of samples

When samples consisting of a great number of corn plants must be prepared for analysis, care must be taken in the proper selection of an aliquot from which the sap is to be expressed. The nitrate nitrogen concentration of various portions of the corn plant and the percentage of nitrate nitrogen found in each portion in relation to the total in the plant are shown in table VII.

The stalk or stem of the corn plant was found to contain 76 per cent. of the total nitrate nitrogen on September 9, while the leaves and sheath contained only 3.75 per cent. Not only was the stalk higher in nitrate nitrogen content than any other part of the plant, but the lower part of the stalk (in this case representing everything from the second node below the ear to the ground) contained 57 per cent. of the total nitrate nitrogen found in the plant. Since the concentration of nitrate nitrogen on a percentage green weight basis exhibits considerable variation in separate parts of the plant, the employment of careful technique in the preparation of the sample is important.

The procedure in this study was to prepare the leaves and stalks separately. Sufficient leaves were ground to provide the 100-gm. sample for the hydraulic press. The stalks were split lengthwise into quarters, five or six quarters from as many different stalks constituting a sample for grinding. After thorough mixing, the 100-gm. samples for the press were weighed from this ground material. Although the seasonal variation in the nitrogen content of the ear was not studied as a part of this problem, a procedure similar to that used for preparing the stalk samples was followed in the determination of the data presented in table VII. When studies are to be

TABLE VII

NITRATE NITROGEN IN SEPARATE PARTS OF PLANT; SAMPLED SEPTEMBER

	NITRATE NITROGEN						
	LEAF AND SHEATH			STALK			
	LEAVES	SHEATH	TOTAL	UPPER	MIDDLE	LOWER	TOTAL
NO_3 N per plant portion (mg.)	3.63	2.09	5.72	3.18	25.88	87.00	116.06
Percentage NO_3 N per plant portion (green weight)	0.00312	0.00252		0.01082	0.02931	0.08987	. . .
Percentage total NO_3 N in plant	2.38	1.37	3.75	2.09	16.99	57.10	76.18

	EAR					SUM TOTAL	STALK		
	HUSK	SHANK	COB	GRAIN	TOTAL		NODE	INTERNODE	TOTAL
NO_3 N per plant portion (mg.)	7.49	1.50	9.27	12.12	30.38	152.16	6.55	18.05	24.60
Percentage NO_3 N per plant portion (green weight)	0.00676	0.00750	0.00728	0.01600	... .	....	0.03195	0.02282	....
Percentage total NO_3 N in plant	4.92	0.99	6.09	7.97	19.97	99.90	26.61	73.39	100.00

made between varieties of corn or on corn receiving different fertility treatments, stalk analysis alone may give sufficient data to indicate any variation in the nitrate nitrogen content of the plant.

When treatments or varieties are replicated several times and samples are collected from the replicated material, it appears necessary to collect all samples from like replicates on the same day. Samples which were collected from replicate plots two days apart showed relatively great difference in nitrate nitrogen content compared with the total amounts present, as shown in table VIII; while the variation between similar treatments sampled on the same day was relatively much smaller.

TABLE VIII

METHOD OF SAMPLING TWO OR MORE REPLICATES

PLOT NO.	NITRATE NITROGEN PER PLANT			
	REPLICATE A SAMPLED SEPT. 16; D SAMPLED SEPT. 18		BOTH REPLICATES SAMPLED SEPT. 23	
1 A	23.5		36.1	
1 D	15.8		35.9	
Average	19.65		36.0	
Difference		7.7		0.2
14 A	19.2		72.3	
14 D	7.3		84.5	
Average	13.25		78.4	
Difference		11.9		12.2
17 A	12.4		32.0	
17 D	12.1		35.8	
Average	12.25		33.9	
Difference		0.3		3.8

Variations in climatic factors may be great within a few days' time. Considerable rainfall between September 18 and 23 probably accounts for the great increases in the nitrate nitrogen content of the later samples from all plots. Light rainfall following a long dry season apparently caused the increase in the nitrate nitrogen content of the "A" replicates from the earlier sample date. The rainfall probably was insufficient to benefit the corn for more than a day or two; consequently the "D" replicates sampled

TABLE IX

EFFECT OF FREEZING AT – 20° C. AND TOLUENE PRESERVATION ON NITRATE NITROGEN CONTENT OF CORN SAP

PLOT NO.	FRESH				FROZEN – 20° C.				TOLUENE				D_A*
	1	2	AV.	DIF.	1	2	AV.	DIF.	1	2	AV.	DIF.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
1	8.26	8.61	**8.44**	0.35	8.40	8.47	**8.44**	0.07	8.40	8.54	**8.47**	0.14	0.03
2	8.12	8.19	**8.16**	0.07	8.12	8.12	**8.12**	0.00	7.98	8.26	**8.12**	0.28	0.04
5	6.16	6.30	**6.23**	0.14	5.88	5.25	**5.57**	0.63	5.88	6.09	**5.99**	0.21	0.66
6	7.14	6.79	**6.97**	0.35	8.05	7.91	**7.98**	0.14	7.35	7.42	**7.39**	0.07	1.01
8	6.58	6.51	**6.55**	0.07	7.28	7.42	**7.35**	0.14	...	...	...	...	0.80
9	7.63	7.84	**7.74**	0.21	7.14	7.14	**7.14**	0.00	7.14	7.28	**7.21**	0.14	0.60
1A	8.19	8.26	**8.23**	0.07	8.19	8.19	**8.19**	0.00					0.04
2A	8.47	8.02	**8.25**	0.45	8.26	7.84	**8.05**	0.42					0.20
5A	5.67	5.74	**5.71**	0.07	5.74	6.09	**5.92**	0.35					0.21
6A	5.25	5.32	**5.29**	0.07	5.25	5.46	**5.36**	0.21					0.07
8A	5.46	5.18	**5.30**	0.24	5.25	5.11	**5.18**	0.14					0.12
9A	6.72	7.07	**6.90**	0.35	6.58	6.65	**6.62**	0.07					0.28
14A	6.79	6.57	**6.68**	0.22	6.44	6.65	**6.55**	0.21					0.13
17A	5.53	5.32	**5.43**	0.21	5.46	5.39	**5.43**	0.07					0.00
19A	5.81	5.95	**5.88**	0.14	5.74	5.88	**5.81**	0.14					0.07
20A	6.72	6.72	**6.72**	0.00	6.44	6.79	**6.62**	0.35					0.10

* D_A = difference between extremes of averages.

two days later were lower in nitrate nitrogen content than the "A" replicates which were sampled on September 16.

PRESERVATION OF SAMPLES

The fact that apparatus sufficient to determine the nitrate nitrogen content of a considerable number of samples at one time is not usually available and would be cumbersome to operate makes the preservation of the sap samples necessary. That both toluene preservation and freezing of the sap at – 20° C. were satisfactory is shown in table IX. Ten-cc. aliquots of sap were pipetted into test-tubes and either frozen at once or preserved in an iced refrigerator after the addition of a few cubic centimeters of toluene to the sample.

The first six samples (plots 1 to 9, table IX) were preserved for one week; the next ten samples (plots 1A to 20A) were preserved for 12 days. Although there is little difference in favor of either toluene or freezing preservation of the samples, the freezing method under long storage would probably be the more satisfactory. Furthermore, the frozen samples are more easily handled, since a slight warming of the test-tube causes the sample to slip easily into the Kjeldahl flask and very little sap remains to be rinsed from the tube.

Summary

1. The SESSIONS and SHIVE aspiration method has been found to be satisfactory for the determination of inorganic nitrogen in plant sap. Slight modifications in quantity of reagent and time of aspiration are necessary for the determination of the inorganic nitrogen content of corn sap.

2. The total inorganic nitrogen content of the corn plant can be determined from an analysis of an aliquot of expressed sap.

3. Because of the extreme variation in nitrate nitrogen content of the separate parts of the corn plant, proper preparation of the sample for analysis is important.

4. Sampling of a series of replicates should be done on the same day to avoid variations in the nitrate nitrogen content caused by changes in climatic factors.

5. Expressed sap samples can be preserved for a time by the use of toluene or by freezing at – 20° C. The latter method is preferred.

OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO.

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RELATION OF HYDROGEN-ION CONCENTRATION OF SOILS TO THE GROWTH OF CERTAIN PASTURE PLANTS

H. P. COOPER

In the course of a study of the ecological conditions influencing the various plant associations found in 546 New York pastures, a large number of pH determinations were made with the quinhydrone electrode. The soil samples were taken from under a number of common species of plants found in these pastures.

It was observed that there is a more or less definite succession of pasture plant associations accompanying the depletion of pasture soil. The types of plants dominating at the various soil fertility levels are also greatly influenced by the climatic conditions as well as by the soil characteristics.

When the timber is first removed from virgin soil, conditions are favorable enough for the growth of Kentucky bluegrass and white clover on practically every well drained soil type in the state. There is usually sufficient nutrient material in the organic residues of the soil for the dominance of crops, like Kentucky bluegrass, which require a high fertility level. The duration of a good Kentucky bluegrass sod in pastures is generally determined by the inherent fertility of the soil.

In the strongly acid soils the available nutrients which have accumulated in their surfaces are soon exhausted and Kentucky bluegrass, which requires a relatively high fertility level, is succeeded by plants tolerant of a lower fertility level. The succession of plant associations accompanying the depletion of pasture soils in regions of New York with cool moist growing seasons may be arbitrarily grouped as follows: (a) Kentucky bluegrass and white clover; (b) Kentucky bluegrass, bent grasses, and white clover; (c) bent grasses and white clover; (d) bent grasses, sweet vernal grass, and white clover; (e) sweet vernal grass; (f) sweet vernal grass and poverty grass; (g) poverty grass; and (h) herbaceous weeds, shrubs, and trees (**1, 3, 4**).

The pH values of soil samples taken from under the vegetation found at the various soil fertility levels are found in table I. Numerous attempts have been made to correlate plant growth with the reaction of soils (**7, 8**). Extended bibliographies will be found in previous papers on this subject (**1, 3, 4**). It is observed that there is no very close correlation between the dominance of various pasture plants and soil reaction. The modal class for Kentucky blue, bent, sweet vernal, and poverty grasses is the same, having a pH range from 5.00 to 5.50. This would suggest that there is some major soil condition other than acidity determining the succession of plants accompanying the depletion of certain soils.

TABLE I

The pH values of soil samples under common pasture plants found in New York

Plant	pH range and frequency distribution										Total
	3.50–3.99	4.00–4.49	4.50–4.99	5.00–5.49	5.50–5.99	6.00–6.49	6.50–6.99	7.00–7.49	7.50–7.99	8.00–8.49	
Kentucky bluegrass											
(*Poa pratensis*)	......	14.0	42.0	75.0	48.0	43.0	43.0	45.0	44.0	5.0	359
Per cent. in each class		3.9	11.7	20.8	13.3	11.9	11.9	12.5	12.3	1.4	100
Bent grasses											
(*Agrostis canina, A. tenuis*)	2.0	15.0	177.0	239.0	147.0	44.0	33.0	12.0	13.0	2.0	684
Per cent. in each class	0.3	2.2	25.9	34.9	21.5	6.4	4.8	1.8	1.9	0.3	100
Red top											
(*Agrostis alba*)			5.0	20.0	18.0	6.0	8.0	12.0	7.0	1.0	77
Per cent. in each class			6.4	25.6	23.0	7.7	10.3	15.4	8.9	1.3	100
Sweet vernal											
(*Anthoxanthum odoratum*)		1.0	8.0	20.0	5.0					...	34
Per cent. in each class		2.9	23.5	58.8	14.7					..	100
Poverty grass											
(*Danthonia spicata*)		1.0	44.0	61.0	49.0	25.0	9.0	4.0		...	193
Per cent. in each class		0.6	22.8	31.6	25.4	12.9	4.9	2.0		.....	100
Canadian bluegrass											
(*Poa compressa*)			2.0	21.0	25.0	10.0	8.0	15.0	19.0	......	100
Per cent. in each class			2.0	21.0	25.0	10.0	8.0	15.0	19.0	.....	100
Red fescue											
(*Festuca rubra*)			8.0	31.0	62.0	31.0	17.0	24.0	16.0	4.0	193
Per cent. in each class			4.1	16.0	32.1	16.0	8.8	12.4	8.3	2.0	100
Creeping bent											
(*Agrostis stolonifera*)	2.0			14.0	14.0	12.0	62.0	61.0	17.0	2.0	184
Per cent. in each class	1.1			7.6	7.6	6.5	33.7	33.1	9.2	1.1	100
White clover											
(*Trifolium repens*)		....	8.0	13.0	10.0	6.0	6.0	1.0		....	44
Per cent. in each class			18.2	29.5	22.7	13.6	13.6	2.3	......	......	100
Total	4.0	31.0	294.0	494.0	378.0	177.0	186.0	174.0	116.0	14.0	1,868
Per cent. in each class	0.2	1.7	15.7	26.4	20.2	9.5	10.0	9.3	6.2	0.8	100

PEARSALL (5) has suggested that the ratios of the various available cations in the soil may be more important than the hydrogen-ion concentration in determining the distribution of plants. It has been suggested by COOPER *et al.* (**1, 2, 3, 4**) that there is a possible relationship between plant growth and the quantity and strength of the available cations and anions present in certain soils. They believe that the oxidation-reduction potential of nutrient materials may influence their availability in soils, also the qualitative order of absorption of the common atomic nutrient ions by plants. If these conditions exist, one would not expect a close correlation between the H-ion concentration of the soil and plant growth. It is the opinion of PIERRE (6) that the percentage base saturation of soils is often more important than H-ion concentration in determining the growth of plants on certain acid soils.

It is often difficult to distinguish between *Agrostis canina* and *A. tenuis* in the seedling stage, as they are commonly found in well managed pastures. These species are found growing together in many of the pastures in certain regions of New York, therefore the pH values for these two species are combined. Velvet bent is apparently very much more abundant than Rhode Island bent in some of the better bent grass pastures. Velvet bent is the predominant grass in many of the very best pastures found in the state.

The data in table I show that the soil samples from under Red fescue and Canadian bluegrass were less acid than the soil from under Kentucky blue, bent, sweet vernal, and poverty grasses. Creeping bent grass was not generally found on strongly acid soils. The modal class for the creeping bent grass is pH 6.50–7.00. This condition was not expected when this study was begun. These data suggest that creeping bent is not so tolerant as some of the other species of bent grass to nutritional complexes characteristic of certain acid soils. Some of the good pastures in western and northern New York contain large quantities of creeping bent grass.

The pH values of soils collected from under some of the common plants encroaching upon depleted pastures are found in table II. White cedar and arbor vitae are very common on pasture soils containing limestone. The modal class is pH 6.50–7.00. Tamarack is also found on soils with a relatively low hydrogen-ion concentration. Mosses, mountain laurel, and white pine are frequently found on strongly acid soils. These data suggest that gray birch, hemlock, and spruce are very tolerant of nutritional complexes characteristic of strongly acid soils.

Summary

1. Data on the pH value of 2317 soil samples collected from under some common plants found in 546 New York pastures are reported. There is a

TABLE II

THE pH VALUES OF SOIL SAMPLES FROM UNDER COMMON PLANTS ENCROACHING UPON DEPLETED PASTURES IN NEW YORK

PLANT	pH RANGE AND FREQUENCY DISTRIBUTION											TOTAL
	3.00–3.49	3.50–3.99	4.00–4.49	4.50–4.99	5.00–5.49	5.50–5.99	6.00–6.49	6.50–6.99	7.00–7.49	7.50–7.99	8.00–8.49	
Paintbrush												
(*Hieracium aurantiacum*)					4.0	10.0		1.0		1.0	1.0	17
Per cent. in each class					23.5	58.8		5.9		5.9	5.9	100
Mosses			9.0	14.0	13.0	3.0	1.0	2.0	2.0			44
Per cent. in each class			20.5	31.8	29.5	6.8	2.3	4.5	4.5			100
Mountain laurel												
(*Kalmia latifolia*)			18.0	36.0	4.0	5.0						63
Per cent. in each class			28.6	57.1	6.0	7.9						100
Beech												
(*Fagus grandifolia*)			8.0	7.0	11.0	5.0	1.0	1.0	4.0	7.0		44
Per cent. in each class			18.2	15.9	25.0	11.4	2.3	2.3	9.1	15.9		100
Gray birch												
(*Betula populifolia*)	4.0	5.0	12.0	17.0	2.0	6.0				1.0	1.0	48
Per cent. in each class	8.3	10.4	25.0	35.4	4.2	12.5				2.1	2.1	100
Arbor vitae												
(*Thuja occidentalis*)												
White cedar												
(*Chamaecyparis thyoides*)				4.0	2.0	11.0	15.0	22.0	6.0	5.0	1.0	66
Per cent. in each class				6.1	3.0	16.7	22.7	33.3	9.1	7.6	1.5	100
Tamarack												
(*Larix laricina*)		1.0		1.0	2.0	5.0	9.0	1.0	1.0			20
Per cent. in each class		5.0		5.0	10.0	25.0	45.0	5.0	5.0			100
White pine												
(*Pinus strobus*)			4.0	18.0	21.0	7.0	3.0	4.0	3.0			60
Per cent. in each class			6.7	30.0	35.0	11.7	5.0	6.7	5.0			100
Hemlock												
(*Tsuga canadensis*)	2.0	2.0	10.0	13.0	7.0	6.0	2.0			1.0		43
Per cent. in each class	4.7	4.7	23.3	30.2	16.3	13.9	4.7			2.3		100
Red spruce												
(*Picea rubra*)	2.0		6.0	4.0	5.0	1.0						18
Per cent. in each class	11.1		33.3	22.2	27.8	5.6						100
Total	8.0	8.0	75.0	119.0	75.0	61.0	34.0	32.0	17.0	17.0	3.0	449
Per cent. in each class	1.8	1.8	16.7	26.5	16.7	13.6	7.6	7.1	3.6	3.6	0.7	100

more or less definite succession of pasture plant associations accompanying the depletion of pasture soils. Kentucky bluegrass requires a fertile soil, and it is likely to dominate on the good soils. As the soil is depleted, bent grasses or red top encroaches upon the Kentucky bluegrass and may dominate. On further depletion of the soil sweet vernal grass may dominate; and finally poverty grass, weeds, and trees represent the dominant type of vegetation.

2. Data from the various grass plant associations accompanying the depletion of pasture soils do not show a close relationship between the soil reaction and the dominance of the various pasture grasses. The modal class for Kentucky blue, bent, sweet vernal, and poverty grasses is the same, ranging from pH 5.00 to 5.50. These data suggest that there is some major soil condition other than acidity determining the succession of pasture plants accompanying the depletion of certain soils.

3. Creeping bent grass was not found on soil with a high hydrogen-ion concentration, which suggests that it is not so tolerant as some of the other species of bent grass to nutritional complexes characteristic of certain acid soils.

4. Arbor vitae and white cedar are common on pasture soils containing limestone. Mosses, mountain laurel, and white pine are frequently found on strongly acid soils. Gray birch, hemlock, and spruce seem to be very tolerant of nutritional complexes characteristic of certain strongly acid soils.

5. It is suggested that a well balanced supply of available cationic and anionic nutrient materials with relatively high oxidation-reduction potentials is much more effective than the hydrogen-ion concentration of the soil in determining the dominance of various pasture plants.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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ISOLATION IN PURE CULTURE OF GREEN ALGAE FROM SOIL BY A SIMPLE TECHNIQUE

C. E. SKINNER

There have been several procedures described for the isolation of green algae in pure culture, free from bacteria and molds. Among the ones most often cited are those of CHODAT (3), SCHRAMM (7), and PRINGSHEIM (6). These methods are essentially alike. Some of the material from which the algae are to be obtained is inoculated into an inorganic nutrient solution. After incubation in sunlight and when growth is apparent, a small amount is inoculated into an agarized inorganic nutrient solution which is incubated in the light. After incubation, when green colonies appear, some of these are carefully picked out of the agar and each one inoculated into a flask of inorganic liquid media. This "enrichment culture method" is repeated until all the contaminating forms are eliminated. Then, instead of planting in inorganic media, the colonies dissected from the agar are planted in media containing dextrose. In case no molds nor bacteria develop and none can be found under the microscope, the culture is thought to be free from bacteria and fungi, and can be maintained in a manner similar to bacterial cultures.

This method is not very applicable to soil, as was shown by BRISTOL ROACH (2, 8), since inoculation of the soil into inorganic media allows a few rapidly growing species to predominate, and so only cultures which grow most rapidly in liquid media are isolated, whereas other species, possibly more numerous, may be repressed or overgrown. Thus a true picture of the soil flora is not obtained. BRISTOL ROACH (8) had devised a method to counteract this defect in part, but in the opinion of the writer it is complicated and time-consuming. A few species were isolated by her method; but, after introducing simplifications and modifications, I was able to obtain about fifty soil strains of unicellular and small filament strains free from bacterial or fungal contaminants in the space of about eighteen months. Never more than one hour a day was consumed (exclusive of the time spent in preparing the media, which was done by a technician); often nothing was done for weeks at a time; and twice the cultures were not touched for a period of more than ten weeks. No special equipment was needed. The method in principle is that followed by BRISTOL ROACH. With the modifications, however, the isolation of algae from soil offers fewer difficulties than the isolation of anaerobic bacteria by the usual somewhat similar technique (5). This point is emphasized, since not a few botanists and bacteriologists have expressed the opinion to the writer that the separation of green algae from bacterial contaminants was a feat requiring the greatest refinement of technique, much patience, and an amount of time greater than most people would wish to give to the work.

The following media were used:

Medium I (Detmer's, diluted to one-third as recommended by Chodat **4**):

$Ca(NO_3)_2 \cdot 4H_2O$	1 gm.
KCl	0.25 gm.
$MgSO_4 \cdot 7H_2O$	0.25 gm.
KH_2PO_4	0.25 gm.
Distilled H_2O	3000 cc.
0.1 per cent. solution of $FeCl_3$	1 cc.

Medium II (Beijerinck **1**):

NH_4NO_3	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$CaCl_2$ (anh.)	0.1 gm.
K_2HPO_4	0.2 gm.
Distilled H_2O	1000 cc.
0.1 per cent. solution $FeCl_3$	0.2 cc.

Medium III (Beijerinck's modified):

Like medium II, but KH_2PO_4 substituted for K_2HPO_4.

When a solid medium is wanted, the solutions are solidified with 1.5 per cent. high grade agar agar. About 10 cc. of the medium was dispensed in each of the test-tubes which, for solid media, preferably possess thin walls, since many of them will have to be broken. If Burri tubes are available, they may replace the test-tubes and need not be sacrificed. All solid media are sterilized for a minimum amount of time at 15 pounds' steam pressure to avoid hydrolysis of the agar.

Twelve tubes of agar medium I or II are melted and cooled to 42°–45°. Into the first are added a few drops of a 10 per cent. (approximate) thoroughly shaken suspension of soil in sterile tap water. The first tube is rotated vigorously or agitated with the pipette to mix it. From the inoculated first tube, 1–2 cc. of the agar are added to the second and thoroughly mixed. This is continued throughout the series, diluting each time with 1–2 cc. of the previously inoculated tube, thus diluting the soil suspension in series. The same pipette may be used throughout, if sterile precautions are observed and the pipette is cleaned out each time by sucking the agar back and forth. The tubes are placed in racks and incubated, if possible, on a north windowsill in summer, and in the winter away from the cold of the window but where they get a few hours of direct sunlight; for instance, on a table near a south, east, or west window. After an incubation period of about one month or more, one or two of the tubes showing isolated, imbedded, green colored colonies is broken and the agar cylinder placed on sterile paper. This cylinder is broken repeatedly rather than cut, as many colonies as possible picked out with a sterile and cooled wire, and each colony inoculated into a tube of medium I or III, preferably the latter. A rather fine wire is used, since it cools more quickly and allows less agar to adhere to it. Although a nichrome wire may be used, platinum is better since it cools more quickly. It is advantageous to get as little of the agar as possible into the solution. These tubes are incubated as before in racks or, if sufficient window space is not available, in tumblers. Other agar tubes may be broken later to obtain slower developing colonies. Each tube of liquid media now usually represents a separate strain of soil algae.

As soon as a green color has developed in the tubes of liquid media, shake agar dilution cultures are again made and incubated. When these show growth, tubes showing a few isolated colonies are selected and the colonies again picked. This time, however, the transplants are put into medium II containing also 0.5 per cent. glucose and 0.2 per cent. peptone. About 22 colonies of each strain are transplanted into this medium and two colonies into inorganic medium I or III.

If bacteria or molds are present in the tubes, growth will be apparent in 2–5 days; if not, the tubes are incubated until algal growth is apparent, when they are transplanted to agarized medium II slants to which 0.5 per cent. dextrose and 0.2 per cent. peptone have been added. If no growth of bacteria or molds becomes evident on the slants, the cultures are probably free from these microorganisms. This may be ascertained by staining the cultures with any bacterial stain and by transplantation to bacteriological culture media. That the algae are pure cultures, free from other species of algae, is probable, but this point may be verified by microscopic examination or by a single cell technique, which is much easier than in the case of bacteria. The purified cultures are maintained on agar slants of dextrose-peptone medium II agar, and any future bacterial or mold contamination may be recognized readily on this medium.

The writer has found that in more than one-half of the trials with the 22 tubes as described, at least one of the tubes was free from bacteria and fungi and showed a growth of algae. However, when all the 22 tubes are contaminated, the two tubes of inorganic solution are allowed to grow in the light, are again diluted out in inorganic shake agar cultures, and colonies are picked and again planted into 22 dextrose-peptone solution tubes and into two tubes of inorganic media. The same procedure is repeated with these until a pure culture is obtained.

In a few cases it has happened that the bacteria which were not eliminated in three or four trials could be repressed by inoculation into media acidified with additional KH_2PO_4. In a few cases the molds could not be eliminated by the methods used and were discarded. Possibly the use of an absolutely inorganic medium with silica gel as the solidifying agent would have been effective. This, however, was not attempted.

Although there is little new in principle in this procedure, it is given to soil biologists in the hope that the simplifications may assist in the isolation of soil algae. Since the writer is not a phycologist, no attempt has been made to identify most of the strains obtained. He will be pleased, however, to supply transplants to any botanist who is interested in the taxonomy of algae.[1] It is generally considered far more difficult to free blue-green than green algae from bacteria, and in this the writer is in hearty accord. So far all such attempts have met with failure, although PRINGSHEIM (6) and others have succeeded in isolating these interesting forms.

It might be mentioned that the use of the different media was not for the purpose of providing variety. Solutions near a neutral reaction are used in agarized media so as to prevent hydrolysis of the agar. DETMER's one-third (medium I) is more acid than BEIJERINCK's (medium II), but medium III (BEIJERINCK's modified) is so distinctly acid as to cause a decided hydrolysis (8). The acid liquid media are used to depress bacterial growth. Inasmuch as medium I contains no NH_4 salt, it may in some cases be used to repress fungous or bacterial growth, since not all molds or bacteria are able to use nitrates. The BEIJERINCK solutions II and III, containing both nitrates and ammonium salts, allow a more rapid growth of algae than solution I; hence both media are used, depending on the condition of the culture. Many other media may be used, the formulae for which may be found in the papers cited. It must be remembered, however, (1) that while acid liquid media tend to repress bacterial growth, excess acid hydrolyzes agar and thus enhances mold development; (2) that nitrates are often inferior to NH_4 salts for algae and also fail to supply available N for certain molds and bacteria; and (3) that media containing NH_4 salts tend to become acid and those containing nitrates tend to become basic, owing to selective absorption of the nitrogen-carrying ions.

DEPARTMENT OF BACTERIOLOGY,
UNIVERSITY OF MINNESOTA,
MINNEAPOLIS, MINN.

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[1] These cultures have been placed in the museum of Prof. E. G. PRINGSHEIM, German University, Prague II, Viničná 3a, Prague, Czechoslovakia, from whom they should be obtained, if desired.

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SUGGESTIONS FOR GROWING MASS CULTURES OF ALGAE FOR VITAMIN AND OTHER PHYSIOLOGICAL STUDY

M. F. GUNDERSON AND C. E. SKINNER

Pure cultures of algae are of great interest to physiologists. By means of them the non-essential nature of sunlight in the formation of chlorophyll was discovered about thirty years ago. This subject has been reviewed several times.[1] More recently WARBURG and his school have made important contributions on the physiology of photosynthesis by use of pure cultures of algae. PRINGSHEIM and his associates have studied, in algae, transformations of organic substances and other physiological phenomena under controlled conditions. CHODAT and his students very early studied the utilization of carbohydrates by green plants (algae) and the effect of nutrition on their morphology. Indeed one may suggest that no higher plants are so suitable for such varied physiological study as are unicellular algae. All bacteria may be eliminated, a thing next to impossible with larger plants; and varied nutritional, light, heat, and other external factors may be studied, one by one, without the chemical changes in the environment inevitable in the presence of bacteria. Then too, such small plants allow one to study large populations and the factor of individual variation ceases to operate. The technique used is essentially bacteriological, and one not trained in such a technique should not expect immediate success. As was emphasized in the preceding paper, however, the technique is not difficult.

The writers wish to suggest another use of pure cultures of algae. Mass cultures of a bacteria-free growth may be grown on a substrate of known chemical composition in light or in darkness. Nutritional or external factors may be varied individually. These mass cultures may be fed to animals on diets and the presence or absence of vitamins determined. The substrate, of course, should be free from the vitamin being studied. Thus the factors necessary for the formation of the various vitamins may be determined. The factors of light and chlorophyll may be separated, since many species of algae form abundant chlorophyll in total darkness in the presence of organic sources of energy. Other factors may also be studied one by one. By introducing certain chemicals or nutrients into the medium one may possibly obtain an insight into the chemical nature of the vitamin itself. Such a study has been initiated at the University of Minnesota, and the first results, now ready, will appear in a later publication. Nonchlorophyllous plants (bacteria) are now being used in the same way.

After various trials, the following method was used for obtaining mass cultures for feeding, and is described in the hope that others may be spared the labor which the writers spent before the method was finally adopted. Growth obtained in the same manner may be used for chemical analysis, but it should be remembered that a slight amount of the substrate is obtained with the growth samples.

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[1] SKINNER, C. E., and GARDNER, CLARA G. The utilization of nitrogenous organic compounds and sodium salts of organic acids by certain soil algae in darkness and in the light. Jour. Bact. 19: 161–179. 1930.

One hundred and fifty to two hundred cc. of the agarized medium desired are dispensed into one-liter flat medicine bottles. For algae the writers used Beijerinck's solution,[2] agar with the addition of 0.5 per cent. glucose. They are sterilized in the autoclave for the minimum time necessary and are allowed to harden after being laid on their sides on a perfectly flat surface. It was found convenient to prepare the media in 10-liter amounts. The agar should be washed repeatedly with alcohol and ether, and the glucose with ether.

After hardening, the flasks are inoculated by adding with a sterile 10-cc. pipette about 5 cc. of a suspension of growth, scraped aseptically from an agar slant into a flask of sterile 1 per cent. NaCl or tap water. It is much more convenient for two persons to do this, one to unplug, flame, and plug the flasks, and the other to do the inoculation. The liquid is shaken over the surface of the medium and the bottles are then laid on their sides in a dark cupboard or incubator. After a little practice, contaminations are infrequent. When growing algae or pigmented bacteria, contaminants can be recognized without difficulty.

After sufficient growth has taken place, the mass of living organisms is washed from the agar. A little water is added and the growth separated from the agar by introducing a brass rod with a flattened end bent at right angles and with rounded edges to avoid cutting into the agar. This growth is washed into large cups which are centrifuged, and the sediment is collected in glass dishes. The supernatant liquid is used again and again in washing off more algal growth from the other flasks. The combined sediment is then dried by directing a current of warm air directly on the growth. It is important that this be done fairly quickly, in order to prevent bacterial growth. Of course, conditions become unfavorable for growth before the algae are completely dry. The final dried growth is ground and used for the purpose desired. If fed as a supplement to vitamin-deficient rats, in the experience of the writers, no difficulty is encountered in getting the animals to eat it. Naturally, all ingredients must be free of the vitamin being studied.

In a similar way bacteria or other microorganisms may be produced in quantity for chemical analyses or for the purpose of feeding. Although more than one rather elaborate and very costly apparatus devised for the purpose has been described, which is much more efficient than the simple method outlined, the writers wish to suggest the above method for those who do not have the more costly apparatus.

Department of Bacteriology and Immunology,
University of Minnesota, Minneapolis.

[2] Beijerinck, M. W. Notiz über *Pleurococcus vulgaris*. Zent. Bakt. II, 4: 785–787. 1898. (NH_4NO_3, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; $CaCl_2$, 0.1 gm.; K_2HPO_4, 0.2 gm.; H_2O, 1000 cc.; 0.1 per cent. solution $FeCl_3$, 0.2 cc.; agar-agar, 15 gm.).

DETERMINATION OF STARCH IN PLANT TISSUES

R. L. SHRINER

Introduction

During a study of the carbohydrate distribution in grape canes, it became necessary to have a method for the estimation of small amounts of starch. A fairly rapid, accurate method yielding easily reproducible results was desired in order that the analysis of a great number of samples could be completed in a short time.

An excellent survey of the literature dealing with analytical methods for starch is given by WALTON (**11**). Of all the methods listed, one described by THOMAS (**9**) for estimating starch in apple spurs seemed most suitable. The fundamental idea of THOMAS's method is the direct determination of the combined reducing power of glucose and maltose produced by the action of diastase on starch under prescribed conditions. This method avoids the destruction of sugars which always takes place if complete hydrolysis with acids is attempted or if acid hydrolysis after diastatic conversion of the starch is used according to the Official Methods (**6**). This fact was shown by THOMAS and confirmed in the present work. THOMAS determined the reducing sugars by means of the colorimetric method of BENEDICT and OSTERBERG (**1**), which involved the reduction of a picrate-picric acid solution to picramic acid. When this method was tested out on pure starch it always yielded high results (table I). The use of mercuric nitrate as a clarifying agent was also unsatisfactory since it yielded a precipitate which was slow in settling and difficult to filter. Moreover, mercuric nitrate exerts an oxidizing influence on sugar solutions (**5**). It is evident that the clarifying agent must be chosen to suit the particular type of substrate obtained from the different plants.

The procedure which was found most satisfactory is as follows:

Procedure

Three gm. of finely ground material (50 mesh), which had previously been extracted with 80 per cent. alcohol in order to remove soluble sugars, are heated to boiling on a steam bath for 30 minutes with 200 cc. water, and then cooled to 37° C. An aliquot portion of a solution of taka-diastase is added so that 0.1 gm. of the enzyme preparation is present. Two cc. of toluene are added and the mixture incubated for 48 hours at 37° C. The mixture should be shaken as often as possible during this digestion. The mixture is heated to boiling, filtered, and the insoluble material washed with hot water. One to 3 cc. of a saturated lead acetate solution are added and the solution diluted to 250 cc. after cooling to 20° C. After shaking thoroughly and allowing the lead precipitate to settle as much as possible the solution is filtered. The excess lead is removed by adding finely powdered disodium phosphate and filtering again. A copper reduction is run on a 50-cc. portion according to the standard MUNSON-WALKER procedure, using the SHAFFER-HARTMANN (8) iodometric method for determining the reduced copper. A blank must be run using 200 cc. water and 0.1 gm. taka-diastase treated exactly like the sample. The difference between the blank and the sample gives the copper reduction value of the digest. This is calculated in terms of glucose from the standard tables. The amount of starch is then obtained as follows:

Calculations

It has been shown (8) that the *ratio* of glucose to maltose in the diastase substrate is 2:1; hence if $g = $ glucose and $m = $ maltose, then $g = 2\ m$ and $m = \frac{g}{2}$ (1).

The average copper reducing value of maltose is 0.61 that of glucose, so if R represents the copper reducing of the diastase substrate expressed in terms of glucose, then $g + 0.61\ m = R$ (2).

By combining equations (1) and (2):

$$2m + 0.61m = 2.61m = R;\ \text{also}\ g + \frac{0.61g}{2} = R$$

$$\textit{or}:\ m = \frac{R}{2.61}\ \text{and}\ g = \frac{R}{1.305}$$

Starch value of maltose = 0.947

Starch value of glucose = 0.90

$$\textit{so}\ Sm = \text{starch equivalent from maltose} = \frac{R}{2.61} \times 0.947 = 0.362\ R$$

$$\textit{and}\ Sg = \text{starch equivalent from glucose} = \frac{R}{1.305} \times 0.90 = 0.689\ R$$

$$\text{Total starch} = Sm + Sg = 0.362\ R + 0.689\ R = 1.051\ R$$

that is: to determine the amount of starch simply multiply the copper reducing value (R) expressed in terms of glucose by the factor 1.051.

Discussion

The preceding method and calculations are based on the following experimental facts:

1. The presence of glucose and maltose in the diastase substrate is in the ratio of 2:1. When the diastatic conversion is carried out as shown, the variation from the 2:1 ratio is very slight, and THOMAS has shown that

these variations (1.8–2.3) do not materially affect the accuracy of the final percentage of starch.

2. The average copper reducing value of maltose is 0.61 that of glucose (**6**). This value varies somewhat, of course, as is evident from MUNSON and WALKER's tables and the choice of 0.61 is made purely arbitrarily since it has been found that this value gives good results.

3. The starch equivalents for glucose and maltose are the ones usually given (**9**).

It will be noted that the factor calculated from the experimental data is quite different from the stoichiometric factor calculated from the following equation:

$$\begin{array}{lcll} 4C_6H_{10}O_5 + 3H_2O & \longrightarrow & 2C_6H_{12}O_6 + & C_{12}H_{22}O_{11} \quad (3) \\ \text{Starch} & & \text{Glucose} & \text{Maltose} \\ 4 \times 162 = 648 & & 2 \times 180 & 342 \end{array}$$

Since the average copper reducing value of maltose is 0.61 that of glucose,

$$\begin{array}{ll} \text{then } 342 \times 0.61 = & 208 \\ \text{for glucose } 2 \times 180 = & 360 \\ & \overline{} \\ \text{sum of glucose and maltose} = & 568 \end{array}$$

$$\text{hence ratio should be } \frac{648}{568} = 1.141 \text{ theoretically.}$$

However, there is no evidence for equation (3). The diastase substrate contains glucose and maltose in the *ratio* of 2 : 1 but this does not mean that four $C_6H_{10}O_5$ units of starch produce two moles of glucose and one of maltose. In fact, the number of moles of glucose and maltose is less than these values and recent work indicates that maltose is not a primary cleavage product of starch but is produced by the synthetic action of the taka-diastase on the glucose. It is also evident from table I that the use of this factor would lead to high results for pure starch. The use of lead acetate as a clarifying agent and disodium phosphate as a de-leading agent is much more satisfactory and more rapid than the use of mercuric nitrate, and it avoids the reduction of any mercury with simultaneous oxidation of the sugars. It is essential that disodium phosphate be used for de-leading, as shown by ENGLIS and TSANG (**4**), and especially so in the present method since the SHAFFER-HARTMANN iodometric titration method for estimating the reduced copper is used. The use of sodium oxalate or sodium carbonate as a de-leading agent is prohibited since both of these interfere with the determination of the reduced copper by this method.

The substitution of the SHAFFER-HARTMANN volumetric method for the colorimetric method has several advantages. In the first place it eliminates the personal error involved in methods which depend on the matching of colors. The starch-iodine endpoint is easily observed. The titrimetric method is also much more rapid, requiring only one-half to one-third the time required for the colorimetric comparison. Although the objections to and disadvantages of the Munson-Walker reduction method are recognized, it has been found that with a little experience this method yields good results. Since the copper reducing ratio of maltose to glucose is only 0.61 while the picric acid reducing ratio is 0.91, it might be expected that this would result in a reduction in accuracy of the method. The data in table I, in which the present method is compared with other methods on a

TABLE I

Data on pure starch

Weight of pure starch taken	Amount of starch found by		
	Diastatic conversion followed by hydrolysis with 0.5 per cent. HCl	Colorimetric according to Thomas	Cu reduction on diastase substrate
gm.	*gm.*	*gm.*	*gm.*
0.2000	0.1599	0.2162	0.2008
0.2000	0.1591	0.2162	0.2003
0.3000	0.2531	0.3563	0.2953
0.3000	0.2476	0.3523	0.2961

TABLE II

Summary of starch determinations in grape cane

Sample no.	Method		
	By hydrolysis with HCl after diastase copper reduction	Colorimetric accordng to Thomas	Writer's method
	per cent.	*per cent.*	*per cent.*
1	1.52	2.79	2.46
2	1.98	3.44	2.61
3	3.84	5.44	4.98
4	2.60	3.86	3.43
5	3.25	4.87	3.93
6	3.35	5.29	4.49
7	5.45	5.93	5.30
8	4.27	4.79	4.72
9	1.61	2.63	2.39
10	4.03	5.26	4.50

sample of purified arrowroot starch, indicate that this factor has had no appreciable effect on the accuracy of the results.

It will be noted (table I) that the colorimetric method yielded high results; the acid hydrolysis (0.5 per cent. HCl) after diastatic conversion yielded low results (indicating destruction of the glucose); while the direct copper reduction yielded results closely approximating the amount of starch taken. Since it has been shown that 2.5 per cent. hydrochloric acid possesses a destructive action on glucose (**3**, **7**, **9**, **10**), 0.5 per cent. acid was used (**9**). This concentration is sufficient to hydrolyze the maltose and any remaining unhydrolyzed dextrins. It was also found that the use of 2.5 per cent. hydrochloric acid gave still lower results than those in table I.

In table II the same relationship between the methods is found to hold when applied to the determination of starch in ten different varieties of grape canes.

Summary

A method for the determination of starch is described which involves: (1) conversion of the starch into glucose and maltose by the action of taka-diastase; (2) determination of the combined reducing value of the glucose plus maltose by the SHAFFER-HARTMANN modification of the MUNSON-WALKER method. A simplified method of calculating the amount of starch from these data is given.

DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF ILLINOIS,
URBANA, ILL.

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MANNOSE AND THE FIRST SUGAR OF PHOTOSYNTHESIS[1]

HARRY F. CLEMENTS

During the past half century, scientific effort has been made to determine the course of photosynthesis. Among the numerous lines of investigation pertaining to this subject, the occurrence and behavior of the simple sugars and sucrose have interested many investigators. Physiologists seem to agree now that the simple sugars are the first sugars formed by plants; and since the pentoses do not occur generally in leaves, the hexoses, glucose and fructose, might logically be the first sugars formed. It is possible that only glucose is the first sugar synthesized and that fructose, as well as all the other plant carbohydrates, arise from it. If this be the case, then it might well be expected that mannose should be formed also, since structurally it is so nearly like glucose and fructose; and, too, whenever glucose is converted by means of its enolic forms to fructose in the laboratory, mannose is produced as abundantly as fructose. In other words, these three sugars when existing under conditions favoring the enolic conversion will exist in equilibrium. To date, mannose has not been found in photosynthetic tissues, but an attempt to find it there has apparently not been made. It is the object of this study to make a rather extensive search for mannose in leaves of many species of plants. It is hoped that the results may be such as to be of value in determining the first sugar of photosynthesis.

In 1903 HÉRISSEY (**3**), after studying mannans and galactans in seeds, concluded that these reserves are digested to form mannose and galactose respectively; but since neither of these sugars was positively identified in juices of living plants, if they exist at all they must exist as intermediate products of hydrolysis and must be changed very quickly to other compounds. Thus although mannan, which after hydrolysis yields mannose and mannitol (a hexahydric alcohol), has been found in many plant parts, mannose itself has never been found except in traces in those parts of plants in which the mannan is stored.

The plants used in this investigation were:

Pteridium aquilinum var.
    pubescens
Pseudotsuga taxifolia
Abies grandis
Pinus ponderosa

Pyrus malus
Pyrus communis
Lupinus ornatus
Pisum sativum
Melilotus officinalis

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[1] Contribution no. 34 from the Botany Department of the State College of Washington.

Pinus monticola
Picea engelmanni
Triticum aestivum
Avena sativa
Tradescantia fluminensis
Allium acuminatum
Veratrum speciosum
Salix sp.
Populus tremuloides var. aurea
Urtica holosericea
Frasera fastigiata
Rumex crispus
Beta vulgaris
Philadelphus lewisii
Heuchera glabella
Rosa spaldingii
Prunus cerasus
Medicago sativa
Geranium viscosissimum
Pelargonium domesticum
Acer negundo
Parthenocissus quinquefolia
Tilia glabra
Althaea rosea
Heracleum lanatum
Syringa vulgaris
Verbascum thapsus
Sambucus coerulea
Balsamorhiza sagittata
Tragopogon porrifolius
Arctium minus
Wyethia amplexicaulis
Helianthus annuus

In this group, the Pteridophyta and the Spermatophyta are represented. Plants belonging to both Gymnospermae and Angiospermae were used. Of the Angiospermae, leaves were collected from plants of 22 families. Not only were plants selected to represent widely separated families, but plants whose soil preferences varied widely were employed.

The leaves of these plants were collected between 1:00 and 2:30 P. M. on successive days, beginning June 12, 1931. Usually six or seven species were taken each day. The leaves were taken into the laboratory and 300 gm. of green material quickly ground in a plate grinder. Sufficient water was added to make the ground mass fairly liquid. The watery material was then brought to a boil and boiled gently for two hours. At the end of this time the liquid was filtered through cheese cloth and cooled. Enough neutral lead acetate was added to clear the solution. After filtration, the excess lead acetate was precipitated with di-sodium acid phosphate and again filtered. The filtrate, which totaled some 300 cc., was reduced under diminished pressure to 20 cc. The pressure was so adjusted that the solution boiled at 35° C. The concentrated solution was cooled under tap water, and whatever precipitate formed was filtered off. To the filtrate, 2.6 gm. of phenylhydrazine hydrochloride and 3.0 gm. of sodium acetate were added. Vigorous shaking of this mixture usually resulted in the complete solution of the added compounds. The samples were then allowed to remain for 24 hours in a refrigerator kept at 1° C. At the end of this period, a search was made for the crystals of mannose phenylhydrazone.

In order to determine the accuracy of the method used, 0.1 gm. of d. mannose was added to 300 gm. of ground leaf material of *Heracleum lanatum, Medicago sativa, Urtica holosericea,* and *Balsamorhiza sagittata* respectively, and thoroughly mixed into each sample. The procedure was followed as just outlined. In each, an abundance of a crystalline precipitate resulted which on recrystallization in 60 per cent. alcohol yielded the platelike, colorless prisms characteristic of the hydrazone of mannose.

Mannose was found to be absent from the leaves of the 42 species of plants used. Since such large quantities (300 gm.) of leaves were used and the extracted solution was concentrated to 20 cc., it is highly improbable that mannose exists even in the slightest traces in photosynthetic tissues, for the method used was found to be sensitive to a 0.03 per cent. solution of mannose.

Although mannose could not be found in these investigations, it may not mean that it is universally lacking in leaves, but it certainly does mean that it forms no part of the general photosynthetic scheme in plants. This being the case, it is probable that fructose in leaves has an origin which is independent of glucose; for unless the stereo-chemical relationships known to exist among glucose, fructose, and mannose are different in plant sap from what they are in pure water, it may be expected that were fructose formed from glucose, mannose should likewise be present. This is not the case.

A number of investigators (**1**) have reported that the occurrence of pentoses is not widespread in photosynthetic tissues. CLEMENTS (**2**) has found sucrose to be absent from leaves during the morning when photosynthesis is active. By a process of elimination, fructose and glucose seem to be the only sugars generally associated with carbohydrate production. That fructose arises from glucose normally in green leaves seems improbable in view of the finding reported herein. Apparently, then, glucose and fructose arise simultaneously as the first sugars of synthesis.

Summary

1. Mannose is found to be absent from leaves of 42 species of plants.
2. Since mannose arises from glucose together with fructose in alkaline solutions, and since mannose is not present in leaf sap. it is concluded that fructose does not arise from glucose in plants but that its origin is simultaneous with that of glucose.
3. By eliminating the pentoses, mannose, and sucrose as sugars not always present when photosynthesis is in progress, it seems probable that glucose and fructose are together the first sugars of synthesis in green leaves.

STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

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BRIEF PAPERS

A PLANT TEST OF THE SUN LAMP[1]

(WITH ONE FIGURE)

In the course of investigations relative to temperature effects in plant growth, it became advisable to adopt artificial illumination as a substitute for difficultly controllable solar heat. This practice involved the issue of spectral adequacy in the case of the ordinary Mazda electric lamp. Tests based upon this point have shown that the young wheat plant is favored in assimilating the nitrate radicle by exposure to the relatively high output of shorter visible and longer ultraviolet radiations emitted by the carbon arc. However, this form is less conveniently controlled for quantitative irradiation than more modern types of lamp.

In view of these considerations it seemed particularly desirable to test the growth responses of plants to the Sunlight (Type S 1) lamp recently developed by the General Electric Company. This lamp is described by LUCKIESCH.[2] A unit of this type was placed at the writer's disposal through combined assistance from the Committee on Radiation of Living Organisms, Division of Biology and Agriculture, National Research Council, and the General Electric Company. Inasmuch as the tomato plant (*Lycopersicum esculentum*) had been found to give rather marked developmental and compositional responses under Vita glass, a form more transparent to solar ultraviolet radiation than common glass, this species was chosen for the present test.

Seedlings of the Livingston Globe variety produced in well manured silt loam were irradiated nine successive nights of 15 hours each, following their emergence from the soil. Sunlight was excluded from the cultures, and the average effective air temperature about the plants was about 22° C. One flat of 60 seedlings was exposed under a 500 watt Mazda C lamp with an intervening cell of flowing water equipped with common glass. A similar flat was exposed to the sun lamp under a water cell constructed from Vita glass. The strongest illumination feasible was utilized, approximating 2000 foot candles. From time to time the distance between the lamp filaments and the upper leaf surfaces was readjusted by the use of a thermopilic photometer to maintain approximate equality of total effective radiation from the lamps.

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[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] LUCKIESCH, M. Artificial sunlight. D. Van Nostrand Co., Inc. 1930.

The appearance of the plants at the close of the test is shown in figure 1. Pigmentation of stem and darker color of leaf under the sun lamp which were conspicuous here were becoming evident on the sixth day. A rather strong phototropic effect under this lamp was also evident. Yields and partial composition of the upper tissues are presented in table I.

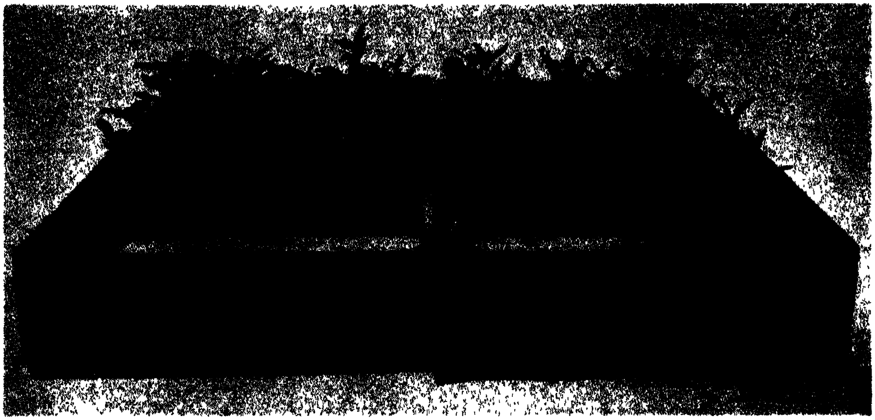

FIG. 1. Tomato seedlings after irradiation nine days from emergence: at left, under Mazda lamp; at right, under sun lamp.

TABLE I

YIELD AND COMPOSITION OF STEMS AND LEAVES IN TOMATO SEEDLINGS WITH RELATION TO IRRADIATION

SOURCE OF RADIATION	WEIGHT OF 60 DRIED PLANTS	LIPIDE CONTENT* OF DRY MATTER		NON-LIPIDE NITROGEN CONTENT OF DRY MATTER	
	gm.	*per cent.*	*gm.*	*per cent.*	*gm.*
Mazda lamp	1.044	3.28	0.034	4.83	0.050
Sun lamp	1.299	2.96	0.038	5.31	0.069

* Ether extract.

Notwithstanding the increased weight of plant under the sun lamp, which might lead one to anticipate more adequate illumination for the requirements of carbohydrate synthesis, the tissue exhibits an increased proportion of nitrogen-containing compounds. This condition is associated with slight depression in the proportion of lipides. As to quantities synthesized, it appears that lipides were favored slightly and nitrogen compounds considerably by exposure of the seedlings to the sun lamp.

Obviously the conditions of exposure solely to the sun lamp are abnormal for plant development. This lamp is intended to provide by relatively brief intermittent exposures the effects of daily exposure to the ultraviolet radiation of sunlight. The results indicate favorable rôles of this form of radiation in relation to the plant. It appears possible to use the sun lamp for limited daily periods in conjunction with major illumination from the Mazda lamp so as to imitate solar radiation of the summer season.—W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin, Madison.*

A DUAL-PURPOSE MICROSCOPE LAMP

(WITH TWO FIGURES)

While in general physiologists make less use of the microscope than do workers in other fields of biology, yet when they are required to make microscopical studies their needs are often extremely exacting. Many times they desire to study cells in the living state, using vital stains or no stain at all where differentiation must depend upon differences in refractive index. Illumination for this type of work must be critical and intensity must be great enough to give bright images after considerable reduction of the condenser aperture.

The writer has constructed a small, compact lamp which has proved very satisfactory for several types of work. It was developed primarily for use with the dissecting microscope (**1**), but has, by the addition of a ground glass and diaphragm and some slight improvements in design, been adapted for use with the compound microscope for both light and dark field work and for photomicrography.

Figure 1 shows the lamp assembled for use and figure 2 shows the various parts. The light source *A*, figure 2, is an Osram 6 volt, 25 watt Nitra globe[1] which fits a standard socket. This is mounted in the rear housing *B* which fits into the front housing *C*. The two condenser lenses *D* and *E* of 5 cm. focal length and 3.8 cm. diameter are mounted in the sleeve *F* by means of the spacer *G* and spring sleeves *H*, and *F* slides into the front tube of *C* and is adjusted by rotating the handle which protrudes through the spiral groove in *C*. A 110 to 6 volt transformer *I* fits in the housing *J*, providing proper current for the Nitra bulb and also serving as a heavy base. The assembled lamp is mounted on *J* by means of a universal joint and swings into several positions, allowing adjustment of height and angle.

The lamp as described thus far makes an excellent illuminator for dissection, providing a beam of intense light. For use with a compound

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[1] Obtainable from E. Leitz, Inc.

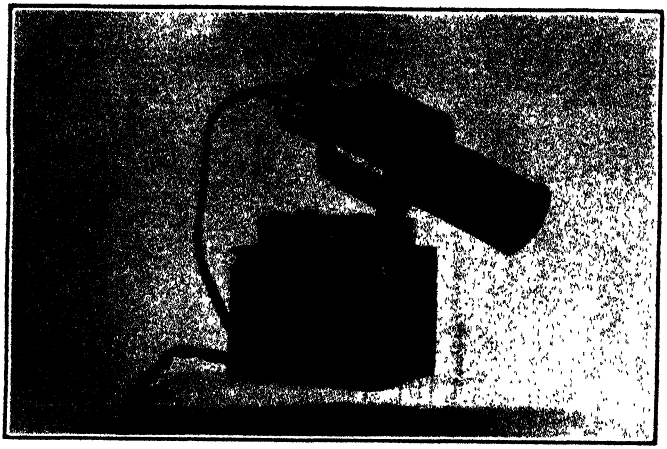

FIG. 1. Microscope lamp with ground glass in position for use with compound microscope.

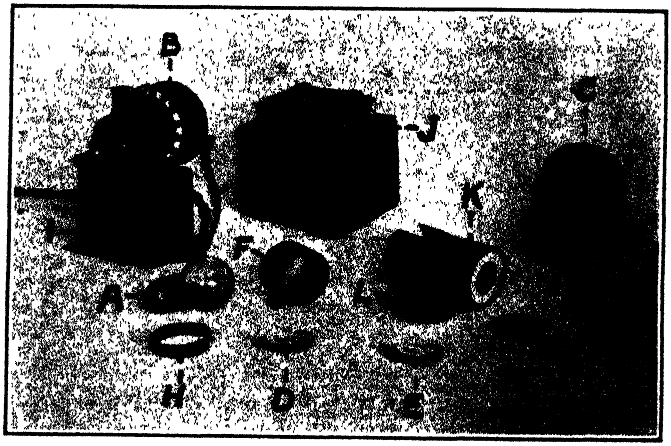

FIG. 2. Microscope lamp disassembled to show the individual parts.

microscope the fitting *K* is slipped on over the front tube of *C*. It bears a ground glass in front of which a hinged door, *L*, with a four-holed diaphragm disk, *M*, may be latched. This disk is pivoted on the back face of *L* and protrudes slightly above it. By brushing the hand lightly along the upper edge of *L* when it is closed, this diaphragm disk may be rotated, centering the different-sized holes with the larger opening in *L*. With the door open and the condenser back, an area 4 cm. in diameter is illuminated for use with the 16-mm. objective. Moving the condenser forward and closing the door *L* gives an intensely illuminated area which may be diaphragmed to spots of smaller diameter for use with higher powered objectives. The ground glass was prepared according to directions by Belling (**2**) and gives a very satisfactory matte.

In using this lamp, it is placed directly in front of the microscope and about 25 cm. from it. The lamp is tilted to point directly at the mirror, and with a slide in place the microscope is brought into focus. Then the condenser of the microscope is adjusted until the outline of the diaphragm on the lamp is in sharp focus in the field of the ocular. The lamp diaphragm should just limit the field of view for low power work, and for very critical high power study it may come considerably within the limits of the ocular diaphragm. If the matte of the ground glass is coarse, the microscope condenser may be slightly raised but the outline of the lamp diaphragm should remain clear.

If a broad color band appears around the edge of the field, the microscope condenser is not achromatic and should be exchanged for one that is. A narrow color band is present with all condensers and serves as a great convenience in centering the microscope condenser. If the latter is out of center this band will vary in color, and by adjusting it so that the color is uniform around the complete periphery of the field, optical centering will be effected.

The addition of a green filter of proper intensity greatly improves this illuminating equipment. The filters are best mounted separate from the lamp so that they can be moved without disturbing the apparatus.

In taking photomicrographs, this lamp may be used either with or without the ground glass. The use of a light filter narrows the transmission band and tends to reduce aberration. The Wratten M filters of the Eastman Kodak Company have proved very satisfactory for this purpose.—A. S. Crafts, *Botany Division, University Farm, Davis, Calif.*

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A USEFUL INSTRUMENT FOR MEASURING HEIGHT GROWTH

(WITH ONE FIGURE)

Lack of knowledge of the tools of the machinist often deprives the botanist and forester of the use of standard stock instruments which are well adapted to their needs. This lack causes them to use makeshift substitutes, or to have "special job" instruments made, which is usually an expensive procedure.

In measuring the height growth of trees at frequent intervals during the past growing season, the writer found a standard machinist's tool known as a drill point gauge to be very useful and satisfactory. The drill point

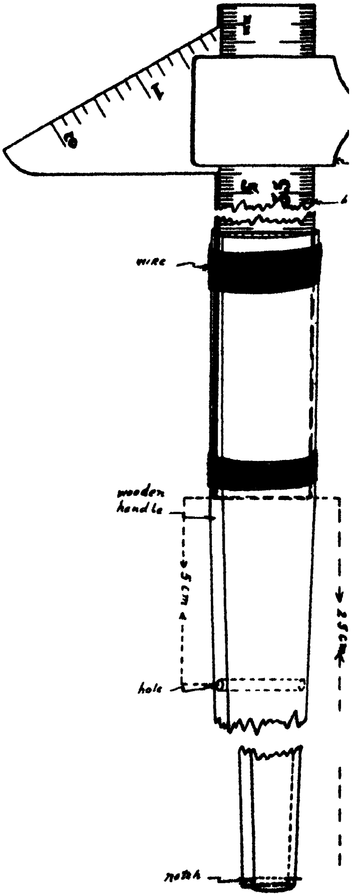


FIG. 1. Drill point gauge showing blade, head, and handle with hole at 5 cm. and notch at 25 cm. from end of steel blade.

gauge consists of a flat, graduated steel blade on which slides a triangular head. The side of the head used in measuring growth extends at right angles from the blade for a distance of about 5 cm. (figure 1).

In actual practice a brass escutcheon nail (number 16 wire, 2 inches long) is driven firmly into the twig (leader in the case of conifers) some distance back from the tip. With this as a base, the distance to the tip of the bud is measured, and after growth starts is remeasured at intervals, the difference between readings giving the amount of growth.

To facilitate holding the base of the blade on the nail, a strong, slotted, wooden handle is slipped over the end of the blade and wired firmly in place. This handle extends 5 cm. beyond the end of the blade and ends in a small notch, which prevents the gauge from slipping off while the gauge head is being moved until it touches the tip of the bud.

If the twig grows vigorously during the season it may grow beyond the length of the blade, in which case a handle 25 cm. or more long may be substituted for the shorter one. There is no difficulty in interpreting the readings when the handles are changed. If some twigs are long and others short, the long handle may be used but with a hole bored 5 cm. from the end of the steel blade to admit the base nail (figure 1).

The drill point gauge as described is made by the L. S. Starrett Company, of Athol, Mass., with heads of two sizes, large and small, and is provided with several lengths of blades, all of which, however, are too short or are provided with unsuitable graduations. However, the 60 cm. blade from another standard tool known as a combination square fits the large-size head perfectly. This blade is graduated in millimeters and half millimeters. It might be advantageous to use a meter blade but such a length is not available as a regular stock tool. Since a wooden handle notched to rest on the base nail is desirable, and since these handles can easily be made in various lengths, the shorter blade is not a serious handicap. The gauge as described can be easily and quickly obtained through local dealers at a cost of about $6.00.

For foresters desiring measurements in 10ths of inches a special blade would have to be made, as regular stock blades in inches are graduated only in 32nds and 64ths.

The writer has used this gauge in making thousands of measurements on the leader growth of various conifers and has found it rapid and yet accurate. With a solid base to measure from and a sharp pointed bud to measure to, growth of as little as a millimeter per day can be accurately determined, provided the set screw is adjusted so that the head slides on the blade without "play." One possible improvement might be the insertion of a tension spring to hold the head from slipping too easily and yet take up the "play" without undue adjustment of the set screw. This gauge should also be of value in measuring height growth in herbaceous plants, using the ground as a firm base from which to measure.

In measuring the thickness of twigs, a small steel caliper rule (75 mm. long) graduated in half millimeters, also a standard stock tool, was found indispensable for accuracy and speed.—RAYMOND KIENHOLZ, *Yale School of Forestry and Connecticut Agricultural Experiment Station, New Haven, Conn.*

NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists has resulted in the election of the following officers:

President: DR. D. R. HOAGLAND, University of California;
Vice-President: DR. C. O. APPLEMAN, University of Maryland;
Secretary-Treasurer: DR. W. A. GARDNER, Alabama Polytechnic Institute.

A very busy season usually confronts the newly elected officers, as there is always an approaching annual meeting, with its many problems and difficulties. The officers will appreciate cooperation on the part of the members of committees, and of the members generally. The prompt performance of each duty as it comes along makes a pleasure of service.

International Botanical Congress.—According to the decision of the Fifth International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive Committee has been formed. President of which is Professor Doctor F. A. F. C. WENT (Utrecht). Professor Doctor J. C. SCHOUTE (Groningen) will act as Vice-President, Dr. W. C. DELEEUW (Bilthoven) as Treasurer, and Dr. M. J. SIRKS (Wageningen) as Secretary. The Committee has decided that the VI Congress will meet at Amsterdam, September 9th to 14th, 1935. Scientific societies are kindly requested to reckon with these dates in planning their own meetings.

Osborne Medal.—The American Association of Cereal Chemists has awarded its THOMAS BURR OSBORNE gold medal to Dr. CLYDE H. BAILEY, Professor of Agricultural Biochemistry at the University of Minnesota, for distinguished contributions in cereal chemistry. Formal presentation of the medal was made at the annual meeting of the Association at Detroit, May 23–26, 1932. Dr. BAILEY has been very active in his chosen field and deserves the honor which has been bestowed upon him. He is a member of the American Society of Plant Physiologists, and gave much helpful advice to the officers of the Society at the Kansas City meeting in 1925, when the plans for the establishment of PLANT PHYSIOLOGY as our official journal were under discussion.

George Janssen.—News of the death of Dr. GEORGE JANSSEN of the University of Arkansas at Fayetteville, a member of the American Society of Plant Physiologists, came as a distinct shock to those who had just recently met him at the meeting of the Society in New Orleans. A sad commentary on the ways of life is provided by the fact that he there served on the Resolutions Committee. A seemingly indefatigable worker, JANSSEN had not been endowed with the best of health, which accounts for his relatively sudden demise at the age of 35 years.

Following his early years spent in South Dakota, JANSSEN obtained his collegiate training at the State College of that commonwealth and received the B.S. degree from that institution in 1921. From 1920 to 1922 he served as Agent of the Cereal Office, United States Department of Agriculture, and in the latter year was granted the M.S. degree for work in agronomy. During this period he became co-author of experiment station bulletins on the culture of potatoes and wheat.

From 1923 to 1926 JANSSEN served as Research Assistant at the University of Wisconsin, in cooperation with the Office of Cereal Investigations of the U. S. D. A. In 1925 he was awarded the degree of Ph.D. at Wisconsin. His thesis, dealing with the relation of seeding date to the physiology and winter-hardiness of wheat, appeared in the Journal of the American Society of Agronomy **21**: 168–200, 444–466. 1929. Since 1926 Dr. JANSSEN had held the position of Assistant Professor of Agronomy at the University of Arkansas. There he had participated in some of the newer phases of work on the functions of potassium in plant nutrition.

To the professional colleagues of Dr. GEORGE JANSSEN the Society expresses its regret for the untimely loss of an enthusiastic member; and to Mrs. JANSSEN, an alumna of South Dakota State College, and the two children, it extends sympathy.—W. E. T.

Endowment.—Every scientific organization which assumes as part of its services the support of publication of research needs a back-log of permanent income from endowments. The reason for this need is especially obvious in times of stress like the present, when the less active and nonproductive support is lost. It is recognized that the best investigators in such an organization are its best assets, for it is the research of such individuals that supplies the commodity for which we are willing to pay. But as it costs on the average of $5.00 for each printed page, a single important paper may cost more than the individual investigator will pay in the form of dues during his entire life. The answer to the problem presented is endowment. The American Society of Plant Physiologists has recognized the need of a strong financial basis for its work. Two of its three endowment funds are fairly well established. There should be a much larger general endowment fund. As a first objective in building such a fund we suggest that $25,000 would provide income equal to 25 per cent.

of the present cost of publication. If this amount were available now, it would not be necessary to delay publication of papers because of lack of space. Everything else about research is endowed except publication. As research becomes useful only upon dissemination and assimilation into the knowledge, culture, and the practices of civilization, the publication of research is the final necessary step before such assimilation can take place. Why should we not have a named endowment for support of publication, if we have named endowments for research itself? This phase of support has been neglected by men of wealth. There is an opportunity for some man of far vision to make an outstanding contribution to research, by establishing endowments to assist in publication under conditions which insure use of the interest solely for meritorious publication.

Minnesota Section.—During the year 1931–1932 the University of Minnesota Section has held eight meetings. The program of addresses before the group was as follows:

Nov. 10, 1931. (a) Physiological relations between stock and scion. Mr. ERNEST ANGELO. (b) Portraits of plant physiologists. Dr. R. B. HARVEY.

Dec. 1. (a) Plant physiology of the vitamins. Dr. G. O. BURR. (b) Physiology of the blueberry. Mr. F. B. CHANDLER.

Jan. 12, 1932. Transformations of iron in the soil. Mr. H. O. HALVORSON.

Feb. 2. Accumulation of dry matter in corn leaves. Mr. K. H. BURNETT, Concordia College.

March 1. Physiological studies of soil algae. Dr. C. E. SKINNER.

April 5. The physiological development of the female gametophyte. Dr. F. K. BUTTERS.

May 3. Relation of seasonal variations to the eradication of weeds. Prof. A. C. ARNY.

June 7. Time of harvest in relation to yield and quality of the crop. Dr. H. K. WILSON.

Purdue University Section.—The Purdue University Section of the American Society of Plant Physiologists held its annual spring dinner meeting on Monday evening May 9, 1932. Guests of the occasion were Dr. H. E. HAYWARD and Dr. E. J. KRAUS of the University of Chicago, Dr. S. H. ECKERSON of the Boyce Thompson Institute, Dr. G. T. NIGHTINGALE of the New Jersey Agricultural Experiment Station and J. P. MARTIN of the Hawaiian Sugar Planters' Experiment Station. Dr. HAYWARD gave a talk on "Some Botanical Problems" in which he reviewed briefly the history of plant morphology, emphasized present deficiencies in our knowledge of ontogeny, and warned against the modern tendency to excessive and premature publications. On the following day the visitors inspected the research work in the plant sciences at Purdue.

Introduction to Phytochemical Research.—A text-book in mimeographed form bearing this title has been prepared by Dr. R. C. BURRELL, of the Ohio State University, Columbus, Ohio. It is especially designed for advanced students in agricultural chemistry, botany, farm crops, horticulture, soils, etc. Although it was prepared especially for his own classes, Dr. Burrell states that a few copies are available, in case students and investigators in other institutions would like to obtain a copy. The price of the volume is $2.00, and Dr. BURRELL will supply it to any one who desires a copy at that price until the edition is exhausted.

Methods of Analysis.—The third edition of this valuable work was issued by the Association of Official Agricultural Chemists some months ago. A number of new chapters have been added, which give methods of analysis of caustic poisons, naval stores, paints, radioactive substances, and eggs and egg products. Other changes are noted as follows: The chapter on vinegar is now included in the chapter on condiments and spices. Gelatin is included under meat. Beers, wines and distilled liquors have all been combined in one chapter. Feeding stuffs are now included as grain and stock feeds. The appendix defines certain fertilizer terms as adopted by the association. The price of the new edition is $5.00 per volume, with 20 per cent. discount in lots of five or more.

Handbook of Plant Analysis.—The Vienna press of Julius Springer has just issued the second volume of G. KLEIN's Handbuch der Pflanzenanalyze, a work of 973 pages. It is divided into two sections, the first of which (pp. 1–204) is devoted to the analysis of inorganic materials, and the second (pp. 205–973), to organic substances.

The inorganic substances are considered in four sections: analysis of the most important cations (K, Na, Ca, Mg, Mn, Fe, Al), and anions (CO_3, Cl, I, Br, P_2O_5, SO_3, SiO_2), and of occasionally occurring ions (As, Cu, Zn, Co, Ni); analysis of inorganic nitrogen compounds; determination and analysis of ash; and gas and micro-gas analysis.

The organic substances are treated in thirteen sections with the following titles: alcohols; aldehydes and ketones; phenols and quinons; the organic acids; phosphoric acid esters of carbohydrate metabolism; lipoids; fats and waxes; polyhydric (sugar) alcohols; simple carbohydrates (mono- and disaccharides); characteristics, determination and preparation of the individual sugars; and polysaccharides (inulin, glycogen, starch). The index of the volume occupies 85 pages (889–973).

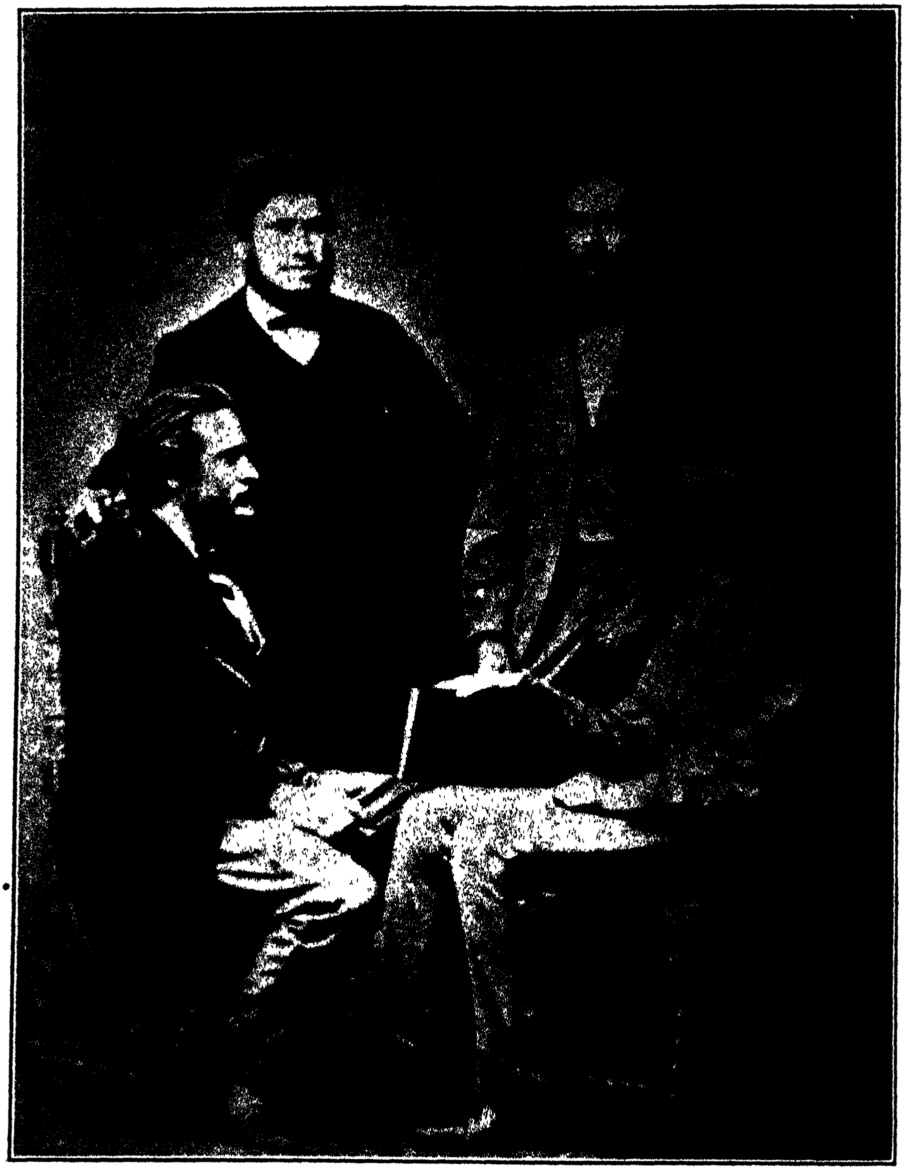
It is a very valuable handbook, and will prove useful in the selection of methods to be used in physiological problems, the solution of which requires the analysis of plant tissues. The price quoted by the publisher for this paper bound volume is RM 96; with cloth binding, RM 99. Orders should be sent to Julius Springer, Vienna I, Schottengasse 4.

Oils and Fats.—During the last two years a second edition of Ubbelohde's Handbuch der Chemie und Technologie der Öle und Fette has been in process of publication. The editor of this important work is HANS HELLER, of Magdeburg. The first, third, and fourth volumes appeared ahead of volume II. The first part of volume II, a book of 824 pages, has now been published with the title Chemie und Technologie der pflanzlichen Öle and Fette. The work is organized on the basis of the kind of oils occurring in plants. The first section (pp. 1–164) surveys the strong drying oils; the second section (pp. 165–382), the weak drying oils; section three (pp. 383–532), the non-drying oils; and the final section (pp. 533–750), the solid fats. In addition there is a brief section on oil cakes, a supplement, and author and subject indexes.

Within each of the main sections, the oils are arranged in the order of the plant families in which they occur, the system of ENGLER and PRANTL being used. This makes it easy to refer to the oils occurring in any family of plants which may be under investigation.

The work is a great storehouse of information, and will be useful to physiologists as well as technological workers with the oils and fats. Orders should be sent to S. Hirzel, Leipzig C 1. The price is quoted as RM 73 for brochure, and RM 79 for a cloth bound copy of this volume on the plant oils and fats.

Soil Conditions and Plant Growth.—The sixth edition of Sir JOHN RUSSELL's famous monograph has been published by Longmans, Green & Co. It has been thoroughly revised, only the introduction remaining practically unchanged. The order of treatment follows that of the preceding edition, but several of the chapters have slightly changed titles. The book has increased to 636 pages. The fact that the first edition in 1912 was a modest volume of 168 pages shows how the subject has grown in 20 years. This book is the best work of its kind in the English language and has been deservedly popular since its first appearance. No one is more masterful in presenting the relations of soils to plant growth than the gifted author of this work. The price of the new volume is 21 shillings.



JOSEF SCHUCH
JULIUS VON SACHS

J. BARANETZKY
HUGO DE VRIES

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EFFECTS OF SULPHUR DEFICIENCY ON METABOLISM IN TOMATO[1]

G. T. NIGHTINGALE, L. G. SCHERMERHORN, AND W. R. ROBBINS

(WITH ONE FIGURE)

Introduction

In connection with studies of protein and carbohydrate metabolism (**26**, **27**, **28**, **29**), and of ammonium and nitrate nutrition (**42**), investigations have been made of effects of deficiency of various essential elements (**29**, **30**, **31**). This paper records the results of a similar series of experiments on deficiency of sulphur. Most of the experiments were conducted with tomato, although apple, narcissus, and asparagus were also used. This work does not include a study nor a review of the significance of sulphur in soils. An extensive review of the literature on this subject is given by JOFFE (**18**), together with the report of his own investigations. Later work on soil fertilization with sulphur-containing compounds is reported by FRAPS (**15**), who likewise reviews much of the literature.

Chemical methods

PLANT FRACTIONS.—The stem tip or upper 25 mm. of stem tissue was in some experiments removed and analyzed separately. The remainder of the stem was divided equally according to length into the two fractions which are termed *lower stem* and *upper stem*. The *petiole* fraction includes all vein and petiole tissue to which the mesophyll of the blade is not directly attached; the smaller veins are included with the other tissues of the *blades* fraction, and have been estimated (**29**) to constitute about 35 to 50 per cent. by volume of that fraction. The analytical sample termed *roots* consists principally of fibrous roots and does not include the small taproot or the region that is intermediate in structure between the root and the stem.

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[1] This work is part of a joint project conducted by the departments of Horticultural Science and of Vegetable Gardening of the New Jersey Agricultural Experiment Station. Journal Series paper of the New Jersey Agricultural Experiment Station, Division of Horticulture.

The Kjeldahl, mineral, and sulphur determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the writers wish to express their appreciation.

The analytical plant fraction of paper-white narcissus which will be referred to as *centers* consists of the unexpanded floral organs and true leaves. Any top growth which was present is included with the centers for analysis and will be termed *centers* and *tops*. The storage scales, together with the disk-like stem of the bulb, are designated *storage tissue*. The entire root system is included in the analytical fraction *roots*. Details concerning the technique employed in separating these bulb fractions are given in another report (**28**).

NITROGEN FRACTIONS.—Fresh tissue only was employed for nitrogenous fractionation. The manner of extraction with water according to the method of CHIBNALL (**5**) has already been described (**29**). The term *protein nitrogen* is used to designate a group or groups of the more complex nitrogenous constituents, and includes both unextracted nitrogen and extracted coagulable nitrogen. The fraction termed by some investigators *total water-soluble nitrogen* is synonymous with *total extracted nitrogen*. In this publication no report is given of this fraction, as such a determination is chiefly indicative of the degree of grinding of the plant tissue, and is of doubtful metabolic significance (**28**). *Soluble nitrogen* as used in this work refers only to that fraction of extracted nitrogen which has been freed of coagulable nitrogen after being boiled and dilute acetic acid added (**5**).

Ammonia was estimated by the method of VAN SLYKE (**43**) on aliquots of the soluble-nitrogen fraction.

Amide nitrogen was determined by the usual procedure on aliquots of soluble nitrogen. It should be mentioned that for the determination of amide nitrogen sulphuric acid has always been employed in these laboratories, because with hydrochloric acid consistent results have not been obtained. Both CHIBNALL (**6**) and VICKERY (**44**) have recently reported that in the presence of nitrates, hydrochloric acid may not be employed in the determination of amide nitrogen, but that sulphuric acid is satisfactory. The humin nitrogen formed was found to be negligible in amount and the filtrate was employed for the estimation of basic nitrogen by precipitation with phosphotungstic acid (**29**).

Alpha amino nitrogen was estimated by the VAN SLYKE method on aliquots of the phosphotungstic acid filtrate (**29**).

Nitrate nitrogen was determined according to the procedure of SESSIONS and SHIVE (**37**) on aliquots of ammonia-free soluble nitrogen.

Other nitrogen was calculated as the difference between soluble nitrogen and the sum of the soluble-nitrogen fractions.

SULPHUR FRACTIONS.—Preliminary trials showed only a slight loss of volatile sulphur from freshly minced stem and blades of tomato that were dried in a current of air at 80° C. The amount lost was too small for quantitative estimation by PETERSON's method (**32**), whereas cabbage leaves subjected to the same treatment yielded a considerable quantity of volatile sulphur. Consequently the determination of total sulphur in tomato tissue was carried out according to Official Methods (**2**), with minced tissue that had been dried rapidly at 80° C. in a current of air and had then been ground as previously described (**28**). For estimation of sulphur fractions, fresh tissue only was employed, because trials showed that the method of drying just described caused a marked decrease in proteins and a corresponding increase in sulphate-free soluble sulphur. The percentage of sulphates was found to be the same in dried as in fresh tissue. However, only in case of narcissus (tables X and XI) was dried material used for sulphate determinations. Aliquots of the protein or coagulum-free extract obtained as described under *nitrogen fractions* were used and the Official (**2**) procedure followed for determination of *sulphates*[2] and total *soluble sulphur*. *Protein sulphur* was computed as the difference between total sulphur and soluble sulphur.

Aliquots of the phosphotungstic acid precipitate obtained from the coagulum-free extract were analyzed for cystine according to the method of PLIMMER (**33**), but the quantity of cystine was insufficient for macrochemical estimation even in large aliquots of tomato stem or blade tissue. The extract had previously been subjected only to the mild acid hydrolysis and sodium carbonate treatment of the amide determination. Analyses for cystine and cysteine were also made by the colorimetric method of SULLIVAN (**38, 39, 40**); pigments present in the tomato-plant tissue interfered with quantitative determinations, although the results clearly indicated that traces of cysteine and cystine were present in the protein-free extract of whole tomato stems.

An attempt was made to adapt the colorimetric nitro-prusside reaction to a quantitative estimation of total S-H sulphur present in the protein-free extract of tomato-plant tissue; but the slight color which occurred was fleeting, and plant pigments interfered.

Microchemical methods

Microchemical tests were made on sections of plant tissue as recommended by ECKERSON (**14**). Some of these tests for sulphur-containing compounds are given later.

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[2] Any sulphites present are included in the sulphate determination. Nitrates at concentrations usually found in tomato were added to nitrate-free tomato stem extract. Recovery of sulphate was not apparently influenced by the added nitrate.

SULPHATES.—One per cent. benzidine in 3 per cent. hydrochloric acid was employed in testing for sulphates. It was the only reaction that was found satisfactory or sufficiently sensitive for testing for sulphates in plant tissue. This test in the presence of sulphates gave glistening white scale-like crystals of benzidine sulphate. Other reactions (**4**) were found more or less satisfactory in testing for sulphates in drops of expressed juice.

SULPHITES.—Extensive trials of many different reagents resulted in only one satisfactory method for testing for sulphites in plant tissue. To thin sections of plant tissue was added a drop or two of 10 per cent. sodium tetrathionate, followed in a few minutes by a drop of 10 per cent. barium chloride. Any sulphites present even in extreme dilution reacted at once to form barium thiosulphate crystals, which look much like starch grains but have a higher refractive index. The crystals often appear in pairs like two mushrooms, with a heavy black border, which is caused by light refraction. This test for sulphites is based upon the fact that salts of polythionic acid react with sulphites to form thiosulphates (**34**), which in turn react with barium chloride to form characteristic barium thiosulphate crystals.

GLUTATHIONE, CYSTEINE.—The nitro-prusside reaction as employed by KOZLOWSKI (**19**) and WHITE (**48**) was used as a test for total S-H or reduced sulphur. It is not, however, specific for either glutathione (**38, 39, 40**) or cysteine, although it has frequently been used by investigators as an index of the glutathione content of plants. Many studies arbitrarily reported as on glutathione are more strictly speaking investigations of the S-H sulphur content of plants. The colorimetric method of SULLIVAN (**38, 39, 40**) was used in making qualitative tests for cysteine on sections of plant tissue, on expressed juice, and on aliquots of protein-free extract of plant tissue.

CYSTINE.—Microchemically cystine was easily identified by its characteristic crystal form, comparative insolubility in water, high refractive index, and chemical reactions.

Experimental methods

Tomato plants (*Lycopersicon esculentum* Mill.) of the variety Marglobe were grown in sifted loam soil in individual 4-inch pots. On May 12, 1931, at the time of commencing experimental treatments, there were available 2500 plants. On that date, 1200 plants were selected for uniformity; the roots of each plant were washed free of soil and the two lower leaves were removed. Two hundred plants were used for initial analysis and 1000 plants were transplanted for experimental treatments to washed quartz sand in new 10-inch clay pots, one or two plants to a pot. The pots were set in shallow enamelware pans. For the period of these experiments no difference in growth was observable between plants that were grown one and those grown two in a pot.

Immediately after being transplanted from soil to sand culture, all of the plants were subjected to nutrient treatments.[3] Some of them received the complete or plus-sulphur, others the minus-sulphur, and a third group the minus-nitrogen solution, as indicated in table I. Also from time to time some of the plants were shifted from one nutrient treatment to another. Each pot received two liters of solution daily, and twice a week each culture was thoroughly flushed with distilled water, after which fresh nutrient solution was applied.

Sufficient iron for subsequent growth was present in the initial plants or possibly as impurities in the salts employed. Likewise, perhaps for the same reason, it was not found necessary to apply boron or manganese; at least the complete-nutrient plants which were allowed to mature grew luxuriantly and produced a heavy crop of fruit. Further, no apparent effect was produced on the minus-sulphur or minus-nitrogen or complete-nutrient plants by the addition of boron or manganese at the rate of one quarter part per million of each.

The plants were grown in a greenhouse at New Brunswick, during the spring and early summer of 1931, under conditions of temperature and humidity suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse, with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 20° C. Each plant was kept pruned to a single stem, and blossoms were hand-pollinated daily; but pollen from flowers of one lot of plants was not used for pollination of flowers of another group. Thirty or more plants of a series were harvested for analysis at 7 A. M. on the several dates indicated in the tables of analytical data.

Results

On May 12, at the time the respective nutrient treatments (table I) were started, the initial tomato plants were about 30 cm. in height, dark green and somewhat soft or succulent; cell walls of stem fibers were little thicker than walls of adjacent parenchymatous tissues; and xylem elements were still comparatively thin-walled. The plants were very low in sugars. The stems contained only 6.10 per cent. dry matter (table II) and were practically devoid of starch except in the endodermis; on the other hand, they were high in organic nitrogen, high in organic sulphur, and contained considerable sulphate and nitrate (tables II and III). Traces of sulphite were also present, particularly in the phloem region of the stem.

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[3] The rather low (29) concentration of nitrate in the nutrient solutions (table I) was purposely maintained in order that there might be no accumulation of nitrates in the tissues of plants which were active in amino acid synthesis. A relatively high percentage of nitrate in a given lot of plants should then be of considerable interpretive value. If tomato plants receive a high external nitrate supply they become practically saturated with nitrate (29), even though they are rapidly synthesizing organic nitrogenous compounds.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED*

	SOLUTION				
	K_2SO_4	$Ca(NO_3)_2$	$Mg(H_2PO_4)_2$	$CaCl_2$	KCl
Plus-S .	0.0045	0.0090	0.0045		
Minus-S .		0.0090	0.0045		0.0045
Minus-N . .	0.0045		0.0045	0.0090	

* Each solution has a total osmotic concentration value of 1.25 atmospheres.

Attention is also called to the fact that these very actively vegetative plants were high in sulphur. Tests of this protein-free aqueous extract showed that the extract contained only a trace of S-H sulphur (nitro-prusside reaction) (**18, 48**). However, rather intense reactions for total S-H sulphur were obtained, but only in the cambium region and in other meristematic tissues, namely, stem and root tips. SULLIVAN'S (**38, 39, 40**) reaction apparently indicated a faint trace of cysteine, likewise limited exclusively to meristematic tissue and not sufficient in quantity to be detected in the extract from whole stems. In view of the fact that according to the nitro-prusside reaction there was apparently much more total reduced sulphur than could be accounted for by the presence of cysteine (SULLIVAN'S method), it would seem not improbable that glutathione was mainly responsible for the positive S-H reaction of active tissues of the plant.

Under the microscope there was observed an occasional naturally occurring crystal of cystine in the comparatively alkaline tissue of the phloem (**9**). The protein-free aqueous extract did not contain a measurable quantity of cystine, as none could be detected by the method of SULLIVAN or by the procedure of PLIMMER (**33**).

PLANTS WHICH RECEIVED COMPLETE NUTRIENT SOLUTION

During the period of these experiments (May 12–July 8), the plus-sulphur or complete-nutrient plants (fig. 1) were moderately vegetative.

TABLE II

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT						
	MAY 12	JUNE 9		JUNE 19		JULY 8	
	INITIAL PLANTS	PLUS-S	MINUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Total sulphate-free S	0.328	0.136	0.112	0.162	0.138	0.166	0.120
Protein S	0.247	0.136	0.091	0.099	0.022	0.166	0.009
Sulphate-free soluble S	0.081	Trace	0.021	0.063	0.116	Trace	0.111
Sulphate S	0.127	0.111	0.011	0.124	Trace	0.173	Trace
Total S	0.455	0.247	0.123	0.286	0.138	0.339	0.120
Total nitrate-free N	2.297	1.315	1.365	1.054	1.255	1.007	1.383
Protein N	1.133	0.915	0.980	0.735	0.709	0.770	0.691
Nitrate-free soluble N	1.164	0.400	0.385	0.319	0.546	0.237	0.692
Basic N	0.280	0.173	0.093	0.071	0.080	0.052	0.110
Amino N	0.561	0.147	0.140	0.137	0.200	0.074	0.276
Amide N	0.272	0.030	0.086	0.049	0.127	0.038	0.158
Ammonia N	0.047	0.020	0.062	0.040	0.100	0.031	0.101
Other N	0.004	0.030	0.004	0.022	0.039	0.042	0.047
Nitrate N	0.279	0.080	0.150	0.060	0.183	0.065	0.301
Total N	2.576	1.395	1.515	1.114	1.438	1.072	1.684
Dry matter	6.100	13.000	14.150	15.250	17.400	18.800	20.350

TABLE III

CONTINUOUSLY PLUS-SULPHUR AND CONTINUOUSLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT						
	MAY 12	JUNE 9		JUNE 19		JULY 8	
	INITIAL PLANTS	PLUS-S	MINUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Total sulphate-free S	0.020	0.018	0.016	0.025	0.024	0.031	0.024
Protein S	0.015	0.018	0.013	0.015	0.004	0.031	0.002
Sulphate-free soluble S	0.005	Trace	0.003	0.010	0.020	Trace	0.022
Sulphate S	0.008	0.014	0.002	0.019	Trace	0.033	Trace
Total S	0.028	0.032	0.018	0.044	0.024	0.064	0.024
Total nitrate-free N	0.140	0.171	0.193	0.161	0.218	0.189	0.281
Protein N	0.069	0.119	0.139	0.112	0.123	0.144	0.140
Nitrate-free soluble N	0.071	0.052	0.054	0.049	0.095	0.045	0.141
Basic N	0.017	0.022	0.013	0.010	0.014	0.010	0.022
Amino N	0.034	0.019	0.020	0.021	0.035	0.014	0.056
Amide N	0.017	0.004	0.012	0.007	0.022	0.007	0.032
Ammonia N	0.003	0.003	0.009	0.006	0.017	0.006	0.021
Other N	0.001	0.004	0.002	0.005	0.007	0.008	0.010
Nitrate N	0.017	0.010	0.021	0.009	0.032	0.013	0.061
Total N	0.157	0.181	0.214	0.170	0.250	0.202	0.342

The initial plants contained practically no starch, and it was not until June 9 that there began to occur considerable starch storage. At about that time many fruits were set. Although the plants were not extremely active nor soft, they were nevertheless dark green and did not lose any of their lower leaves during the period of the experiments. The rate of growth, however, was much slower than that of less fruitful plants that received a complete nutrient solution with three times the concentration of nitrates.

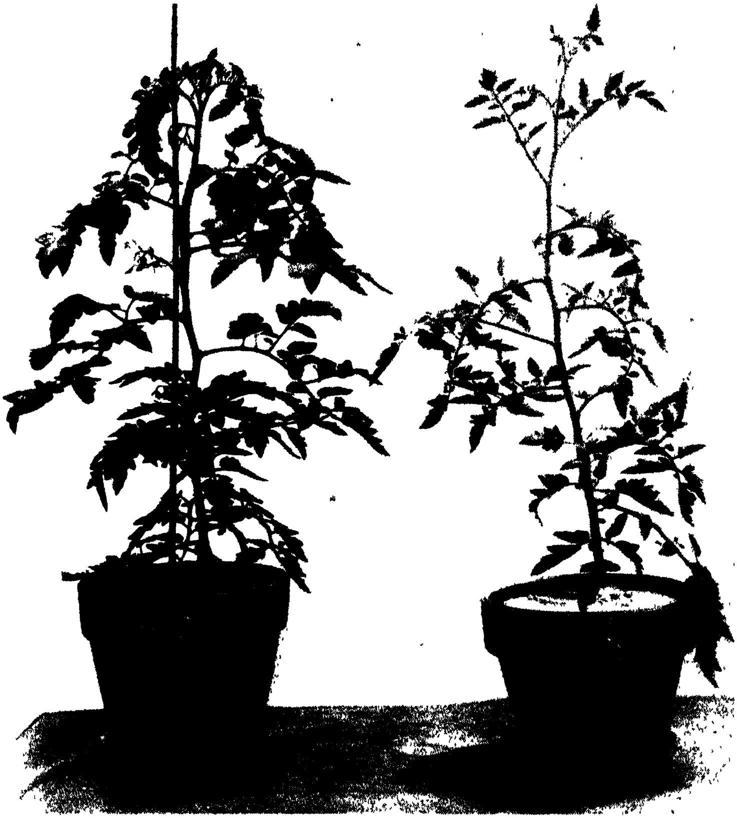

FIG. 1. Tomato plants, June 23, 1931: from left to right, plus-sulphur and minus-sulphur. Compare relative diameter of stems of the two plants. Note that sulphur-deficient plant is so stiff and woody that no stake is required to support it; that it is lighter in color; and that a single fruit has developed whereas fruits have not yet set on the plus-sulphur plant.

On July 8, stems of the fruitful, moderately vegetative plants that were supplied with the complete nutrient solution (table I) contained the following percentage of carbohydrates on a dry weight basis: reducing sugars 4.20, sucrose 4.35, and starch and dextrin 12.17. The moderately low concentration of nitrogenous constituents is indicated in tables II and III.

The percentage of organic sulphur and sulphates was about half that found in the initial plants. Further, most of the sulphate-free sulphur was in the form of protein (tables II and III). Microchemical tests also indicated that there was much less S-H sulphur than in the initial plants. Strong, positive reactions for reduced sulphur, however, were obtained in the vicinity of stem and root tips and in the cambium region, especially of the upper half of the stem.

SULPHUR-DEFICIENT PLANTS

Symptoms of sulphur deficiency developed very gradually, but by June 9 the upper half of the stem of the minus-sulphur plants was very small in diameter and produced no axillary shoots. The total stem length, however, was equal to or greater[4] than that of the plants supplied with the complete nutrient solution (fig. 1). The lower leaves especially, and by July 8 even the upper leaves, were yellowish green with purple veins. The newly formed leaflets were of small area and were spaced far apart on the rachis. Stems were stiff and woody, and the few fruits formed were set and matured early (fig. 1). The minus-sulphur plants looked as if they had been gradually but not completely limited as to their nitrogen supply. This appearance was borne out by the root systems, which had the general character of those usually produced by low-nitrogen plants (**20**, **26**, **29**). The roots were somewhat extensive, but of small diameter because there was practically no cambium nor secondary thickening. Cells did not show any deformity nor injury, however, as in calcium deficiency (**31**).

Macrochemical analyses for essential mineral elements showed that sulphur deficiency did not materially affect the concentration of most of the mineral elements. The concentration of calcium was somewhat high, however, caused apparently by heavy deposits of calcium oxalate, especially in the phloem. The phloem of the minus-sulphur plants was about pH 5.6 as compared with 7.0 in the complete-nutrient series. Other mineral deficiencies have been shown (**10**, **30**) to result in plant tissue of comparatively low pH and high concentration of calcium oxalate. There was no accumulation of any other mineral element; in fact, computed on a dry-weight basis, analyses of July 8 show that the percentage of potassium in the stems of the plus-sulphur plants was 2.98 as compared with 1.99 in the sulphur-deficient series. The lower figure, however, represents a concentration of potassium that is more than adequate for good growth of tomato (**30**). The lower concentration is probably associated with the fact that the minus-sulphur plants had little meristematic tissue. Active tissue is notably high in potassium (**30**).

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[4] The limit of ability of the sulphur-deficient plants to continue stem elongation was not determined. A few specimens were retained until August 1, and even at that time stems were still increasing in length, although the last-formed 30 cm. of stem was not more than 2 mm. in diameter.

When analyzed on July 8, the stems of the minus-sulphur plants contained the following percentage of carbohydrates computed on a dry weight basis: reducing sugars 6.32, sucrose 7.00, and starch and dextrin 14.79. The anatomical structure also corresponded closely to that of typical nitrogen-deficient high-carbohydrate plants (**20**, **30**). The stems had no active cambium except in and near the stem tip; the walls of both internal and external fibers were extremely thick; and there was a very high proportion of thick-walled xylem and collenchyma tissue.

Nitrates were not low; in fact, when the last harvest was made (tables II and III) the stems of the sulphur-deficient lot contained nearly six times the concentration of nitrate found in the complete-nutrient plants. Total organic nitrogen also was consistently a little higher in the minus-sulphur group; this was caused mainly by accumulation of basic, amino, and amide nitrogen. Ammonia also was high (tables II and III). The percentage of total organic sulphur was not materially different in the two groups of plants, but in the plus-sulphur series nearly all of it was protein; whereas in the plants lacking an external sulphate supply there was little protein sulphur but much soluble sulphate-free sulphur. This non-protein water-soluble organic sulphur gave negative tests for cysteine, cystine, and glutathione. Its form was not determined. The extremely small stem tip of the sulphur-deficient plants appeared, however, to give a very faint S-H reaction (nitro-prusside test).

The concentration of sulphur in different parts of the plant is indicated in tables IV and V.

SULPHUR-DEFICIENT PLANTS SHIFTED TO PLUS-SULPHUR NUTRIENT TREATMENT

A few seconds after sulphur-deficient plants were supplied with the complete nutrient solution, sulphates were observed adsorbed on the outer pectic layers of the root hairs, and almost immediately could be detected within them. Five minutes later sulphates appeared in the fine roots, and after six hours large quantities of sulphate were found in the base of the stem and smaller quantities in the remainder of the stem and in the leaves.

The appearance of sulphites in different parts of the plants was almost simultaneous with that of sulphates, although the maximum concentration

TABLE IV

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS

SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT						
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S		DRY MATTER	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S	PLUS-S	MINUS-S
Tip of stem	0.227	0.131	0.189	0.416	0.131	7.89	17.26
Upper stem	0.152	0.111	0.131	0.283	0.111	17.10	19.00
Lower stem	0.119	0.125	0.096	0.215	0.125	20.50	21.70
Fibrous roots*	0.318	0.238	0.463	0.781	0.238	9.02	10.20
Upper petioles	0.221	0.020	0.279	0.500	0.020	11.95	10.32
Lower petioles	0.177	0.103	0.485	0.662	0.103	11.80	11.20
Upper blades	0.387	0.152	0.747	1.134	0.152	17.00	16.80
Lower blades	0.261	0.214	1.871	2.132	0.214	15.50	17.75
Flesh of ripe fruit	0.149	0.106	0.063	0.212	0.106	6.92	5.94
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141	.	..

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

TABLE V

CONTINUALLY PLUS-SULPHUR AND CONTINUALLY MINUS-SULPHUR TOMATO PLANTS
SULPHUR FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER
PLANTS HARVESTED FOR ANALYSIS JUNE 30, 1931

MATERIAL	TREATMENT				
	TOTAL SULPHATE-FREE S		SULPHATE S‡	TOTAL S	
	PLUS-S	MINUS-S	PLUS-S	PLUS-S	MINUS-S
Tip of stem	0.018	0.022	0.015	0.033	0.022
Upper stem	0.026	0.021	0.022	0.048	0.021
Lower stem	0.024	0.027	0.020	0.044	0.027
Fibrous roots*	0.029	0.024	0.041	0.070	0.024
Upper petioles	0.026	0.002	0.034	0.060	0.002
Lower petioles	0.021	0.012	0.057	0.078	0.012
Upper blades	0.066	0.026	0.066	0.193	0.026
Lower blades	0.040	0.038	0.290	0.330	0.038
Flesh of ripe fruit	0.010	0.006	0.005	0.015	0.006
Seed of ripe fruit†	0.228	0.141	0.021	0.249	0.141

* Computed on ash-free basis.

† Computed as percentage of air-dry weight of seeds.

‡ Minus-S plants did not contain sufficient sulphate to be detected by macrochemical analysis (2).

of sulphite was not reached until about 24 hours following the shift to plus-sulphur treatment.

Six hours after absorption of sulphate the roots gave faint but positive reactions for S-H sulphur; somewhat later much stronger S-H reactions occurred, particularly in the cambium and phloem regions and in all tissues of the tops that were not entirely mature. Some, but only a small part, of the reduced sulphur was cystein (SULLIVAN reaction); and therefore by inference (page 567) it may be suggested that most of the reduced sulphur was glutathione.

Seventy-two hours after the shift to plus-sulphur treatment (June 23, tables VI and VII) there was found a decrease in nitrate and an increase in organic nitrogen. Accompanying the decrease in nitrate, strong reactions for nitrite were obtained in the then more alkaline phloem region of roots and tops. Tables VI and VII also show that following the shift to complete nutrient there occurred a marked and very rapid increase in percentage of protein sulphur. Total organic sulphur showed no increase on a percentage basis. This is probably because the plants increased rapidly in volume immediately following the shift. On an absolute amount basis there was unquestionably an increase in total organic sulphur.

The response to an application of sulphates was extremely rapid. The plants had turned much darker green within twelve hours. Even the lower yellow leaves turned green during that time, and by the end of the second and third day there was a marked increase in diameter of newly formed stem and in area of newly developing leaves.

TABLE VI

Minus-sulphur tomato plants shifted to plus-sulphur treatment
Sulphur and nitrogen fractions of whole stems expressed as percentage of dry matter and dry matter expressed as percentage of green matter

Fractions determined	Date and nutrient treatment		
	June 19	June 23	June 30
	Minus-S	Minus-S to plus-S*	Minus-S to plus-S*
Total sulphate-free S	0.138	0.093	0.121
Protein S	0.022	0.052	0.097
Sulphate-free soluble S	0.116	0.041	0.024
Sulphate S	Trace	0.017	0.025
Total S	0.138	0.110	0.146
Total nitrate-free N	1.255	2.253	1.066
Protein N	0.709	1.667	0.617
Nitrate-free soluble N	0.546	0.586	0.449
Basic N	0.080	0.111	0.097
Amino N	0.200	0.273	0.200
Amide N	0.127	0.130	0.082
Ammonia N	0.100	0.082	0.033
Other N	0.039	−0.010	0.037
Nitrate N	0.183	0.077	Trace
Total N	1.438	2.330	1.066
Dry matter	17.400	16.900	15.520

* After June 19, some of the minus-S plants were subjected to plus-S treatment.

TREATMENT IN DARKNESS

The plants which received the nutrient solution lacking nitrogen (minus-nitrogen plus-sulphur) were typical yellow, stunted, woody plants, very low in nitrogen and high in carbohydrates. After twelve days of continuous darkness there was as usual (**26**, **29**) a marked drop in carbohydrates, a decrease in protein and a corresponding increase in amino and amide nitrogen (tables VIII and IX). Total nitrogen decreased in percentage because the plants increased greatly in volume and green weight, although there was no external nitrogen supply. The stems showed an increase in length of 15 to 20 cm., and many new leaves expanded during the interval of twelve days without light. The temperature was about 20° C. In the nitrogen-deficient plants during the darkness period, catabolism seems also to have been the predominant phase, not only of nitrogen but also of sulphur metabolism (tables VIII and IX).

It may further be noted that the minus-nitrogen plants did not accumulate large quantities of sulphate, even though before the shift to darkness the sulphate-free soluble sulphur fraction was high (tables VIII and IX) as compared with that of the complete-nutrient plants (tables II and III). This fraction appeared to contain a trace of S-H sulphur but no cystine. Following the period of darkness, newly developed tissue of the minus-nitrogen plants gave apparently more intense S-H reactions.

TABLE VII

MINUS-SULPHUR TOMATO PLANTS SHIFTED TO PLUS-SULPHUR TREATMENT

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	DATE AND NUTRIENT TREATMENT		
	JUNE 19	JUNE 23	JUNE 30
	MINUS-S	MINUS-S TO PLUS-S*	MINUS-S TO PLUS-S*
Total sulphate-free S	0.024	0.016	0.019
Protein S	0.004	0.009	0.015
Sulphate-free soluble S	0.020	0.007	0.004
Sulphate S	Trace	0.003	0.004
Total S	0.024	0.019	0.023
Total nitrate-free N	0.218	0.381	0.165
Protein N	0.123	0.282	0.096
Nitrate-free soluble N	0.095	0.099	0.069
Basic N	0.014	0.019	0.015
Amino N	0.035	0.046	0.031
Amide N	0.022	0.022	0.013
Ammonia N	0.017	0.014	0.005
Other N	0.007	-0.002	0.005
Nitrate N	0.032	0.013	Trace
Total N	0.250	0.394	0.165

* After June 19, part of the minus-S plants were subjected to plus-S treatment.

The treatment in darkness of sulphur-deficient plants resulted in etiolation of newly expanded leaves but did not appear to affect in slightest degree the rate of stem elongation nor any other noticeable phase of growth as compared with other minus-sulphur plants which were subjected to the seasonal light conditions of the greenhouse during the same period. Carbohydrates decreased materially in the minus-sulphur plants during the 12-day period without light, and there was some indication of catabolism of nitrogenous materials (tables VIII and IX). The same tables of data, however, indicate no material change in percentage nor quality of sulphur fractions.

TABLE VIII

Minus-nitrogen and minus-sulphur tomato plants shifted from daylight to continuous darkness

Sulphur and nitrogen fractions of whole stems expressed as percentage of dry matter and dry matter expressed as percentage of green matter

Nutrient treatment and date

Fractions determined	Minus-N (plus-S)		Minus-S (plus-NO$_3$)	
	June 6	June 18	June 19	July 2
	Normal light	Part of June 6 plants after 12 days' darkness	Normal light	Part of June 19 plants after 12 days' darkness
Total sulphate-free S	0.154	0.118	0.138	0.154
Protein S	0.117	0.068	0.022	0.035
Sulphate-free soluble S	0.037	0.050	0.116	0.119
Sulphate S	0.071	0.138	Trace	Trace
Total S	0.225	0.256	0.138	0.154
Total nitrate-free N	0.828	0.853	1.255	1.683
Protein N	0.657	0.531	0.709	0.820
Nitrate-free soluble N	0.171	0.322	0.546	0.863
Basic N	0.053	0.060	0.080	0.174
Amino N	0.082	0.164	0.200	0.395
Amide N	0.029	0.107	0.127	0.120
Ammonia N	None	Trace	0.100	0.107
Other N	0.007	–0.009	0.039	0.067
Nitrate N	None	None	0.183	0.257
Total N	0.828	0.853	1.438	1.940
Dry matter	15.550	12.740	17.400	12.530

Certain other experiments are considered in the discussion that follows. Results of cooperative studies with Dr. Eckerson on sulphate and nitrate reduction are also included.

Discussion

External responses.—It has been found (**31**) that if seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen

(**20, 29**), phosphorus (**10**), or potassium (**30**), the effects of nitrogen or phosphorus deficiency are evident in a comparatively short time, but conspicuous symptoms of lack of potassium (**30**) are not usually apparent until much later. Although symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of the plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost leaves and tip of the stem are rather dark green and may remain so for a considerable period. On the other hand, calcium-deficient tomato plants (**31**) are distinctive in appearance, in that the upper half of the plant is yellow rather than green, and the lower half instead of being yellowish is rather dark green. The lower leaves often remain green even after death of stem tips and complete loss of chlorophyll from the upper half of the plant.

TABLE IX

MINUS-NITROGEN AND MINUS-SULPHUR TOMATO PLANTS SHIFTED FROM DAYLIGHT TO CONTINUOUS DARKNESS

SULPHUR AND NITROGEN FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

NUTRIENT TREATMENT AND DATE

FRACTIONS DETERMINED	MINUS-N (PLUS-S)		MINUS-S (PLUS-NO$_3$)	
	JUNE 6	JUNE 18	JUNE 19	JULY 2
	NORMAL LIGHT	PART OF JUNE 6 PLANTS AFTER 12 DAYS' DARKNESS	NORMAL LIGHT	PART OF JUNE 19 PLANTS AFTER 12 DAYS' DARKNESS
Total sulphate-free S	0.024	0.015	0.024	0.019
Protein S	0.018	0.009	0.004	0.004
Sulphate-free soluble S	0.006	0.006	0.020	0.015
Sulphate S	0.011	0.018	Trace	Trace
Total S	0.035	0.033	0.024	0.019
Total nitrate-free N	0.129	0.109	0.218	0.211
Protein N	0.102	0.068	0.123	0.103
Nitrate-free soluble N	0.027	0.041	0.095	0.108
Basic N	0.008	0.008	0.014	0.021
Amino N	0.013	0.021	0.035	0.049
Amide N	0.005	0.014	0.022	0.016
Ammonia N	None	Trace	0.017	0.013
Other N	0.001	-0.002	0.007	0.009
Nitrate N	None	None	0.032	0.032
Total N	0.129	0.109	0.250	0.243

TABLE X

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13*

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.150	0.200	0.280	0.255	0.220	0.220
Sulphate S	0.130	0.130	0.720	0.075	0.140	0.780
Total S	0.280	0.330	1.000	0.330	0.360	1.000
Total nitrate-free N	2.940	1.330	3.075	3.340	1.990	4.925
Nitrate N	None	None	None	None	Trace	0.845
Dry matter	11.300	30.080	6.030	10.000	27.270	6.470

* Results have been reported of analyses of these bulbs for carbohydrate and nitrogenous fractions (28).

TABLE XI

PAPER-WHITE NARCISSUS BULBS GROWN IN SAND CULTURE FROM NOVEMBER 16 TO DECEMBER 13

SULPHUR AND NITROGEN FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER

FRACTIONS DETERMINED	NUTRIENT TREATMENT AND PART OF PLANT					
	MINUS-N (PLUS-S)			COMPLETE (PLUS-NO_3)		
	CENTERS AND TOPS	STORAGE TISSUE	ROOTS	CENTERS AND TOPS	STORAGE TISSUE	ROOTS
Total sulphate-free S	0.017	0.060	0.017	0.026	0.060	0.014
Sulphate S	0.015	0.039	0.043	0.007	0.038	0.050
Total S	0.032	0.099	0.060	0.033	0.098	0.065
Total nitrate-free N	0.332	0.400	0.185	0.334	0.543	0.319
Nitrate N	None	None	None	None	Trace	0.055

Sulphur-deficient tomato plants, however, are easily distinguished from those lacking calcium. In the sulphur-deficient plants of these experiments the stem tips of the former, although small in diameter, were not dead nor even injured, and the upper portion of the plant was darker green than the lower. In some respects these plants were similar to plants deficient respectively in nitrogen, phosphorus, and potassium, in that the stems and petioles were stiff and woody, anthocyanin was conspicuously present, and the foliage was light yellowish green, particularly on the lower half of the plant. They exhibited an apparently distinctive and remarkable capacity for stem elongation (fig. 1). Many of the plants lacking sulphur had even longer stems and were taller than those which received the complete nutrient solution. Yet the stems were much more woody, and, as shown in figure 1, were of extremely small diameter.

Low-nitrogen plants (**9**, **20**, **29**) never exhibit such a growth response unless they are subjected to some such treatment as darkness, shading (**26**, **29**), or heavy pruning (**20**); and apparently phosphorus- (**10**) or potassium-deficient tomato plants exhibit rapid stem elongation only following or accompanying carbohydrate depletion, which often occurs only shortly before death of the plant.

PROTEOLYSIS.—The breaking down of proteins to simple water-soluble organic nitrogenous compounds as amino acids and asparagine, and the reutilization of these and similar compounds in the development of new tissues, is a process in plants that commonly follows or accompanies increase in moisture content and decrease in percentage of dry matter and stored carbohydrates (**8**, **26**, **29**). In seed germination there is available a conspicuous example of this process. There are present conditions of abundant moisture, and in the seed there occur continual decrease in stored carbohydrates or fat and rapid breaking down and utilization of storage proteins.

Nitrogen-deficient tomato plants are high in carbohydrates and obviously low in total nitrogen (**9**, **20**, **29**). Further, much of the nitrogen consists of complex relatively immobile storage proteins (**29**). Breaking down of these proteins apparently does not occur except when preceded or accompanied by loss of carbohydrates, as when the plants are placed in continual darkness (**29**). There then occur rapid stem elongation, decrease in complex storage proteins, and increase in percentage of the simpler water-soluble forms of organic nitrogen.

Tomato plants lacking an external potassium (**30**) or phosphate (**10**) supply are, except during the last stages of growth, high-carbohydrate plants; and there is little proteolysis or vegetative extension unless the plants are shaded, placed in darkness, or otherwise treated to decrease the carbohydrate reserves. However, shortly before death of potassium- or phosphate-deficient plants, there occurs a decided decrease in carbohydrates, accompanying which there is, apparently, considerable breaking down and utilization of protein and fairly rapid stem elongation. Death of the plant within a few days may be caused, due in part to deficiency of proteins of the right quality rather than to lack in total organic nitrogen

(**10**, **30**, **31**). Similar conditions may obtain in calcium-deficient plants, except that effects of lack of this element (**30**) are so sudden and drastic that even if carbohydrates are low, the period or extent of proteolytic activity is necessarily very limited.

Sulphur-deficient plants were at all times very high in carbohydrates (page 575); yet the stems of these plants (fig. 1) increased in length, although not in diameter, fully as rapidly as the plants which received the complete nutrient solution. Analyses (tables II and III), however, show that as compared with the complete-nutrient plants, the minus-sulphur group contained on July 8 more than twice the concentration of soluble organic nitrogen and were especially high in amino acids, amide, and ammonia. Further, the organic sulphur in the complete-nutrient plants was nearly all in a complex protein form; whereas, in the minus-sulphur series, it was mainly water-soluble, protein-free organic sulphur, only a very small part of which was cysteine or glutathione.

The initial tomato plants, which were strongly vegetative and actively growing, contained much soluble organic sulphur and gave a strong S-H reaction (page 570). This is in striking contrast to the quality of the soluble organic sulphur of the minus-sulphur plants, which included practically no S-H sulphur. The low concentration of soluble organic sulphur in the only moderately vegetative plus-sulphur plants is not entirely without precedent.[5] AITKEN (**1**) analyzed fresh blade tissue of five different grasses and states that the water-soluble "protein" fraction did not contain sulphur. It should be mentioned, though, that his method of harvesting for analysis resulted in an analytical sample of grass blades which apparently did not include active basal growing tissues. Likewise in apple (**22**, **36**), in *Fagus* (**7**), and in *Salix* (**36**), limited data give some suggestion that sulphur in these plants may be in large part insoluble, especially in mature leaves and storage tissue.

As usual, the plants which received sulphate but no nitrogen in the nutrient solution (minus-nitrogen plus-sulphur series) were very high in carbohydrates, and were making no measurable growth when exposed to seasonal light conditions of the greenhouse; but during the period of darkness accompanying decrease of carbohydrates, the stem increased in length by 15 to 20 cm., protein decreased, and the concentration of soluble organic nitrogen approximately doubled (tables VIII and IX). The changes in sulphur fractions are less, but indicate definitely a marked decrease in sulphur-containing proteins. (Stems only were analyzed; soluble organic sulphur formed proteolytically was presumably translocated to other organs.)

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[5] Drying plant tissue may result in a marked increase in soluble organic sulphur and a corresponding decrease in protein sulphur. Various workers have used dried plant tissue in making analyses for these fractions, but the results would appear impossible of interpretation so far as protein and soluble organic sulphur are concerned.

In contrast it would seem that the sulphur-deficient tomato plants were very actively breaking down sulphur-containing proteins, even under conditions of extreme carbohydrate accumulation. The effects of treatment in darkness on the minus-sulphur plants are in complete harmony with these results. When minus-sulphur plants were placed in darkness, there was a marked drop in carbohydrates but no perceptible change in rate of stem elongation as compared with minus-sulphur plants continually in the light, and no material change in concentration or quality of sulphur fractions (tables VIII and IX). Expressed on a green-weight basis, it is seen that changes in nitrogenous fractions were also slight, although, owing to loss in dry matter, the percentage of nitrogen as well as of sulphur shows an increase.

Proteolytic activity in plants (**8**, **26**, **29**) seems generally to be intimately associated with decrease in storage carbohydrates. Obviously, however, proteolysis in the sulphur-deficient tomato plants was not limited by the presence of a high concentration of carbohydrates. An explanation is not apparent, but it was this capacity on the part of the sulphur-deficient tomato plants which made possible extensive reutilization of organic sulphur.

Whether or not sulphates may be formed proteolytically is uncertain, although there is some evidence to indicate that this may occur (page 591).

COMPOSITION IN RELATION TO ANATOMY.—Tomato plants have previously been grown in a deficiency of nitrogen (**20**, **29**), phosphorus (**10**), potassium (**30**), and calcium (**31**). At various stages of growth, and under different conditions of environment and nutrition, the concentration of carbohydrates has been found to vary widely. Accumulation of carbohydrates, whatever the cause, has been found to be associated with thick cell walls in xylem, phloem region, and cortex; whereas a low concentration of carbohydrates has been found to be associated with comparatively thin walls in these tissues. Similar conditions have been reported by WELTON (**47**) for oats and wheat. Tomato plants grown in a deficiency of sulphur are no exception to this generalization. The minus-sulphur plants of the present experiments were very high in carbohydrates; even the pith cells of the stem tip were thick-walled, although not so thick as the conductive elements of the xylem, fibers of the phloem region, and collenchyma of the cortex. It would seem that the thickness of cell walls is intimately associated with the supply of available carbohydrates, and only indirectly (with the possible exception of calcium[6]) with the supply of various essential elements obtained from the soil.

On the other hand, the characteristics of the protoplast appear to be rather definitely modified by nutrient treatment. Plants low in nitrogen, but with opportunity for abundant photosynthetic activity, have little meristematic tissue but are not injured. A tomato plant may, under advantageous conditions, remain alive for at least two years without external application of nitrogen. The protoplasm, where present, gradually becomes less dense but is highly refractive and does not look opaque. Proteinaceous materials of the protoplasm may be extensively reutilized in the formation of new meristematic tissue; but this apparently occurs, as already mentioned, only when accompanied by a decrease in carbohydrate reserve.

This description applies equally well to minus-sulphur tomato plants, with the single exception that in sulphur deficiency, even though carbohydrates are high, sulphur-containing nitrogenous constituents of the protoplast are constantly being reutilized for the development of progressively smaller stem and root tips. In either nitrogen or sulphur deficiency, proteinaceous protoplasmic material is limited in amount, but there does not appear to be radical disintegration of the protoplast, as is found in phosphorus (**10**) and calcium (**31**) deficiency, nor deposition of the abnormal granular proteinaceous particles frequently observed in plants lacking potassium (**30**), phosphorus (**10**), or calcium (**31**).

It would seem from the results of these experiments with tomato, and from the work of others (**16**, **17**, **18**, **46**, **48**), that cysteine and glutathione or similar compounds are essential constituents of actively dividing cells. At least, meristematic tissues were not observed that did not contain higher concentrations of S-H sulphur than adjacent mature cells. It should be emphasized, however, that many other materials are equally essential. In fact, as already mentioned, sulphur deficiency in tomato was less drastic in its effect than limitation of other essential elements.

NITRATE ASSIMILATION AND CARBOHYDRATE ACCUMULATION.—Some tomato plants were grown with no external supply of either sulphate or nitrate. They were shifted to a solution containing nitrate but no sulphate, and the plants absorbed nitrate instantly. This experiment demonstrates that for nitrate absorption the presence of sulphate in a solution is unnecessary. The plants lacking sulphate and nitrate in their nutrient solution soon became exhausted of the nitrate initially present in the plant. Sulphate, however, was retained as such, and, following complete assimilation of nitrate, did not materially decrease in concentration. As might be anticipated, it was found practically impossible to obtain plants simultaneously deficient in sulphate and nitrate. However, absorption of nitrates does not appear to have been a limiting factor in the minus-sulphur tomato plants of these experiments, as they were consistently much higher in nitrate than the plants which received the complete nutrient solution (tables II and III).

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[6] Calcium deficiency in algae has been said (35) to prevent the formation of the middle lamella. In tomato (31), however, lack of calcium did not prevent the early formation of the middle lamella for which calcium apparently may not be directly necessary. Calcium pectate at later stages may, however, be essential. Especially in the fibrous roots of tomato, there was found a secondary effect of calcium deficiency that resulted in dissolution of the middle lamella and separation of cells.

Nitrates accumulated in the sulphur-deficient plants because the assimilation or synthesis of nitrates to amino acids and other organic nitrogenous compounds was greatly inhibited. ECKERSON (**13**) found that the tomato plants lacking sulphur were low in reducase (nitrate reducing material); the reducase was maintained at low level, allowing continuous but slow reduction of nitrate. Also in a few hours following the shift from minus- to plus-sulphur nutrient solution, the plants gave strong reactions for nitrites; and eleven days after receiving sulphate there was a marked decrease in carbohydrates. Carbohydrate accumulation is not unfavorable to nitrate reduction; in fact, typical low-nitrogen plants are very high in carbohydrates, and especially high in reducase activity (**9**). Accumulation of carbohydrates was not caused by the fact that sugars could not be translocated, nor accumulation of starch by the fact that digestion to sugars was seriously limited. When the plants were placed in darkness, starch was freely hydrolyzed to sugars. Starch and sugars were also present in very large quantities in all parts of the plant, even in stem and root tips. Obviously, translocation of carbohydrates could not have been more complete.

One of the principal uses of carbohydrates, however, is in protein synthesis. Carbohydrates and nitrates (tables II and III) undoubtedly accumulated in the sulphur-deficient plants because there was little synthesis of amino acids or other proteinaceous materials. In this respect, the low-sulphur plants are similar to tomato plants lacking phosphates (**10**) or potassium (**30**), although the response differs in degree (**13**).

NITRATE AND SULPHATE ASSIMILATION.—The assimilation of nitrates in plants (**9**) involves reduction to nitrates and ammonia, following which there is oxidation of sugars and synthesis of amino acids. In the tomato this process appears to be carried on in the more alkaline phloem region of roots and tops. In certain other plants, such as apple (**11**), narcissus (**28**), and asparagus (**26**), the reduction of nitrates is restricted mainly to the fibrous roots; and under favorable conditions of growth, nitrates are seldom found in the tops of these plants because they are synthesized to amino acids and other organic nitrogenous compounds in the roots.

An attempt was made to determine whether sulphate and nitrate assimilation were in any respect similar. ECKERSON found that the minus-sulphur tomato plants were very high in material that reduced sulphates to sulphites. Likewise, when the minus-sulphur plants were shifted to plus-sulphur treatment, sulphites appeared in considerable quantity, especially in the phloem of the tops and to a less extent in similar tissue of the roots. Shortly following the appearance of sulphites, unusually strong reactions were obtained for S-H sulphur, including cysteine. Glutathione also was probably present. The S-H sulphur reactions were largely restricted, however, to the phloem and cambium and to the meristematic tips of stem and roots, a condition that has frequently been observed by others (**16**, **17**, **19**, **48**). No cystine was observed. Undoubtedly there were present other unidentified compounds of soluble organic sulphur such as possibly methionine, a comparatively recently discovered sulphur-containing amino acid (**3**, **25**). There was certainly much soluble organic sulphur in the minus-sulphur plants (tables II and III), even more than before shifting to plus-sulphur treatment; but practically none of it was in the form of cysteine, glutathione, or cystine.

In tomato, therefore, sulphate and nitrate reduction seem in certain respects to be similar, in that plants deficient in sulphur are most active in sulphate reduction, and plants deficient in nitrogen are especially active in nitrate reduction. The region of reduction in both cases is the phloem, and may take place in either roots or tops.

It has been pointed out that in asparagus nitrate reduction takes place in the roots and that nitrates are not usually found in the succulent actively growing stems or spears. Some spears were analyzed and found to contain, on a green-weight basis, 0.036 per cent. sulphate sulphur and traces of sulphite. The spears contained no nitrates. Some potted asparagus plants were placed in a chamber in darkness at 10° C. As usual (**27**) this temperature prevented assimilation of nitrates and they appeared in the spears, but there was no increase in concentration of sulphates. A field-grown apple tree was examined and found to contain nitrates in the fibrous roots only, yet sulphates and sulphites were found in roots, twigs, petioles, and leaves. Likewise, ECKERSON found that in sulphate reduction the tops of apple trees were even more active than the roots, although the reverse was true for nitrate reduction. A sample consisting of twigs and petioles was found to contain, on a green-weight basis, 0.018 per cent. sulphate sulphur, but no nitrates. It is apparent from tables X and XI that narcissus contains nitrate in the roots only, whereas sulphate is present in all parts of the plant.

In these plants, therefore, sulphate assimilation does not appear to be restricted to the roots. Most of the assimilation of nitrates, however, takes place in the roots.

GROWTH OF TOMATO AS ASSOCIATED WITH CONCENTRATION OF NITRATE AND SULPHATE.—Tables IV and V show that the leaves and fruits of the minus-sulphur plants were comparatively low in sulphate-free sulphur. This is not true of the stems and roots, however, especially on a percentage of green-weight basis. Likewise, the data of tables II and III show that the whole stems of minus-sulphur plants were, at all times during the experiment, practically as high in concentration of total organic sulphur as the plants which received and contained an abundance of sulphate.

The sulphur-deficient plants certainly were not low in total soluble organic sulphur (tables II and III) obtained apparently exclusively through the breaking down of proteins (page 583). However, in spite of the high concentration of total soluble organic sulphur, the plants contained practically no cysteine or glutathione. If these or other essential sulphur-containing materials are formed chiefly through sulphate reduction and not proteolytically, it would seem to explain the apparent need for maintaining in the tomato plant, during its period of active growth, a somewhat high concentration of sulphate.

Repeated experiments (**20**, **29**, **30**) involving nitrogen nutrition with nitrates as the sole source of nitrogen show that, for vigorous growth of tomato, there must be maintained in the plant a high concentration of nitrates. The concentration must be far in excess of that which will be assimilated or the plant does not grow vigorously. Nitrates in themselves, however, are not essential for growth; a vigorous nitrate-free tomato plant may easily be obtained by maintaining a lower percentage of ammonium (**42**). It may be that in nitrate or sulphate nutrition of tomato, a considerable quantity of the unelaborated ion must be present to furnish the plant at sufficient rate an adequate amount of certain intermediate products of protein synthesis. In this connection it may be pointed out that HAMMETT (**17**) has shown that low concentrations of S-H compounds in a nutrient solution greatly accelerated growth of roots of seedlings of *Zea mays* and *Phaseolus vulgaris*. It appears probable that in plant nutrition S-H sulphur bears a relationship to sulphate in some respects similar to that of ammonium to nitrate.

There appears to be no information available as to the possible physiological value of an abundance of sulphate[7] and nitrate in tomato. It is certain, however, that lack of sulphate and nitrate cannot be a limiting factor in the synthesis of organic compounds for which nitrogen and sulphur are necessary, so long as sulphate and nitrate are present in abundance. The initial plants, which were high in sulphate and nitrate (tables II and III), gave exceptionally strong S-H reactions, contained some cystine (page 570), and for complete-nutrient plants were comparatively high in sulphate-free soluble sulphur. There are, however, analyses (**18**, **32**, **41**) tending to show that the sulphate content of plants may be low, especially when the plants are nearly mature (**41**). Whether or not a strongly vegetative tomato plant might be obtained that was low in sulphate seems doubtful. It would have been worth while to have determined effects of giving tomato plants a nutrient solution containing no sulphate but instead a low concentration of S-H sulphur continually supplied. Chemicals in sufficient quantity for such nutrient treatment were not available, however. At least it is clear that the tomato plants of these experiments decreased greatly in vegetative activity before there was extreme depletion of sulphate (fig. 1, tables II and III).

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[7] Sulphates do not appear to be held by proteins or other ampholytes of tomato as tenaciously as some other ions, as for example calcium (**31**), nitrate (**42**), or ammonium ions (**42**). Electrodialysis (**23**) of tomato stem tissue failed to remove more sulphate than the usual method (**5**) of aqueous extraction at approximately pH 5.6.

On the other hand, high-protein narcissus bulbs (**28**) containing no nitrates, and with no external source of nitrogen, may grow vigorously for months with a nitrogen supply obtained solely through the utilization of storage proteins. The dormant narcissus bulbs which were studied contained, however, considerable quantities of sulphate (tables X and XI).

It was thought that seeds might be obtained which were free of sulphate and that with them a test might be made to see whether cysteine or other S-H compounds were formed proteolytically. Soy beans were employed, and, although the dormant seeds contained insufficient sulphate for a macro-determination (**2**), the benzidene reaction indicated traces of sulphate. There appeared to be practically no S-H sulphur in the dry seeds. Three hours after integuments had been broken and the seeds had been moistened with distilled water, there were noticeable quantities of sulphate and sulphite (benzidine and tetrathionate reactions respectively, page 568). Fairly strong reactions for S-H compounds were also obtained at that time, particularly in the region of the hypocotyl and epicotyl. The germinating seeds were watched at frequent intervals for a period of five days, but there was no noticeable change in concentration of sulphite or S-H sulphur. But at the end of the five-day germination trial, the quantity of sulphate sulphur in the seeds was found by macro-analysis (**2**) to be 0.006 per cent. This percentage figure is based upon the original air-dry weight of the seeds.

So far as the observations on reduced sulphur are concerned, these results are in agreement with those of VIVARIO and LECLOUX (**45**). The question as to whether S-H compounds were formed synthetically from sulphites or wholly or in part through proteolysis may not be answered at this time. Nitrates appear never (**9, 26, 27, 28, 29**) to be formed proteolytically, yet phosphates and probably ammonium may be formed from the breaking down of phosphatides (**10**). The presence of sulphatides (**21**) has not been demonstrated in plants. However, catabolic changes in organic sulphur-containing compounds would appear to be a possible explanation of the appearance of sulphates in the germinating seeds of soy beans.

In this connection attention may be called to the fact that traces of sulphate (tables II and III) were present in the minus-sulphur tomato plants. Even as late as July 8, an occasional crystal of benzidene sulphate formed on addition of the benzidene reagent to sections of the stem tips of the sulphur-deficient plants. There was, however, no sign of disorganization of the protoplast. In low-phosphorus tomatoes, breaking down of phosphatides to phosphates is accompanied by definite injury to the cells, and death soon follows (**10**).

Summary

1. Symptoms of sulphur deficiency in tomato developed slowly in plants which lacked an external sulphate supply. The plants looked as though they had been gradually but not completely deprived of nitrogen. The lower leaves were yellowish green, the stems were hard and woody, and the roots were extensive. Both roots and stems were of very small diameter.

2. These characteristics may also be exhibited by tomato plants deficient respectively in nitrogen, phosphorus, or potassium. The sulphur-deficient tomato plants had, however, a remarkable capacity for stem elongation; and although the stems were woody and thin they increased in length, but not in diameter, as rapidly as the stems of the complete-nutrient plants.

3. The sulphur-deficient tomato plants were extremely high in carbohydrates, and contained much more nitrate than the plants which received the complete nutrient solution.

4. Carbohydrates and nitrate accumulated in the minus-sulphate treated tomato plants because reduction of nitrates and oxidation of sugars was comparatively slow although not entirely inhibited.

5. Digestion of starch and translocation of sugars took place freely in minus-sulphate tomato plants.

6. Cell wall thickness in tomato plants seems to be more intimately associated with carbohydrate content than with any other single factor of nutrition. The high-carbohydrate, minus-sulphate tomato plants had very thick cell walls and a relatively high proportion of fibers and lignified tissue.

7. Protoplasm of the sulphur-deficient tomato looked much like that of plants lacking nitrogen; that is, the protoplasm was limited in amount but not noticeably injured as in deficiency of phosphorus, calcium, or potassium.

8. Cysteine specifically, probably glutathione, and possibly other S-H compounds were present in meristematic tissue and were especially high

when complete-nutrient tomato plants contained an abundance of sulphate and nitrate.

9. Associated with low content of S-H sulphur in the minus-sulphate tomato plants there was practically no active cambium. Roots and stems were thus of small diameter, but they increased in length as a result of cell division in the apical meristems of stems and roots. The meristematic tissues contained faint traces of S-H compounds.

10. Proteolysis is usually accompanied by decrease in reserve carbohydrates. The minus-sulphate tomato plants were at all times extremely high in carbohydrates, yet complex proteins were rapidly broken down to soluble organic compounds of sulphur, to amino acids, to asparagine, and to ammonia. This proteolytic activity resulted, however, in little if any S-H sulphur.

11. Although the tomato plants lacking an external sulphate supply were not very low in percentage of total organic sulphur, much of it was water-soluble; whereas the organic sulphur of the complete-nutrient tomato plants was mainly in a complex protein form. The soluble organic sulphur in the minus-sulphur plants contained practically no cysteine, glutathione, or cystine. The form of sulphur was not determined.

12. Limited data give some evidence to indicate that sulphate and ammonium may be formed proteolytically. It is known that phosphatides break down in part to phosphates and probably ammonium.

13. In tomato, sulphate is reduced to sulphite and apparently to S-H sulphur in the comparatively alkaline phloem region of roots and tops. This is also true of the reduction of nitrates to nitrites and ammonium.

14. In apple, narcissus, and asparagus, the region of nitrate reduction is largely confined to the fibrous roots. Sulphate reduction in these plants takes place in the roots to some extent, but mainly in the tops.

New Jersey Agricultural Experiment Station,
New Brunswick,
New Jersey.

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INFLUENCE OF POTASSIUM NITRATE ON NODULE FORMATION AND NITROGEN FIXATION BY CLOVER[1]

E. W. Hopkins, P. W. Wilson, and W. H. Peterson

Introduction

The unique position which the Leguminosae occupy in the plant world has made them particularly inviting subjects for investigation. The specialized structure arising from association with benevolent symbionts enables this group of plants to develop independently of the supply of nitrogen present in the soil. Not only do leguminous plants with associated bacteria require no added fixed nitrogen, but also the peculiar fact was long ago observed that nitrogenous compounds adversely affect this so-called symbiotic relation. Under these conditions there may be present large numbers of effective strains of the nodule bacteria, and yet the plant appears to resist their invasion or to make little use of its nodules. Thus it would seem that if both sources of nitrogen are available the plant prefers to feed upon fixed nitrogen in the soil. This subject has been investigated repeatedly, and several hypotheses have been proposed to explain this peculiar condition. However, the investigations have considered chiefly the effect of nitrate treatment on nodule formation, since that is the form in which plants usually take up their nitrogen, and because of its wide occurrence in soil. A second important form of soil nitrogen, *viz.*, ammonia, has been given far less attention; while organic nitrogen sources have been almost entirely neglected.

The major part of the work that has been done in this field has dealt with the effect of sources of nitrogen on the number of nodules. Inquiries that deal with the effect on the nitrogen fixation process are rare and often open to criticism. Thus the use of open pots for growing the plants is not satisfactory since growth of algae and other microörganisms would account for part of the nitrate that disappears. Obviously plants grown under bacteriologically controlled conditions are desirable if a satisfactory nitrogen balance is to be made. To grow plants free from contaminating organisms, it is necessary to adopt precautions that result in smaller plants than those grown in open pots. This leads to much smaller nitrogen uptake and the nitrogen balances are more likely to be affected by analytical errors. In spite of these objections it appeared desirable to investigate the formation of nodules and nitrogen fixation with plants grown under controlled conditions.

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A second point that does not seem to have received sufficient consideration is the question whether the effects noted are due to the concentration of nitrogen at any one time, or to the total nitrogen added. It is possible that different results would be obtained if the nitrogen were added periodically rather than at one time.

RAUTENBERG and KÜHN (**11, 12**) appear to have been the first to observe that vetch (*Vicia faba*) formed no root nodules in solutions containing ammonia or nitrate nitrogen, while plants grown in nitrogen-free solutions possessed abundant nodules. DE VRIES (**13**) noted that red clover plants grown in nitrogen-rich solutions developed only a few nodules, as compared with plants without access to combined nitrogen. FRANK (**5**) found that horse-manure extract prevented formation of nodules by peas grown in a soil which had been ignited.

Historical review

It is not possible to give here a detailed review of the numerous papers which discuss the effect of nitrate treatment on nodule formation. A summary of these papers is given in the monograph by FRED, BALDWIN, and McCOY (**6**). However, certain papers may be reviewed here, as they offer details not given in this monograph. FLAMAND (**4**) has made an extensive study of the concentrations of nitrate which inhibit nodule formation. Plants were grown in SACH's solution with various nitrates added. With peas (*Pisum sativum*), KNO_3, $NaNO_3$, and NH_4NO_3 were effective in dilutions of 1/10,000 in preventing nodule formation, while with $Ca(NO_3)_2$ a 1/2000 dilution was required. Vetch (*Vicia narbonensis*) exhibited no nodules in $NaNO_3$ 1/2000; KNO_3 1/10,000; $Ca(NO_3)_2$ less than 1/10,000; and NH_4NO_3 1/20,000. Horse bean (*Faba equina*) was somewhat less sensitive to some nitrates and more sensitive to others; KNO_3 1/10,000; $Ca(NO_3)_2$ 1/20,000; $NaNO_3$ 1/2000. $(NH_4)_2SO_4$ inhibited nodule formation on *Pisum sativum* and *Faba equina* at a dilution of 1/10,000 and on *Vica narbonensis* at 1/20,000.

WILSON (**14**) has studied the effect upon soybeans of many alkali and metal nitrates. An amount of these nitrates which had been found to have no injurious effect on the plant was added to 208 gm. of soil. The nitrates used were as follows: $Ca(NO_3)_2$, KNO_3, NH_4NO_3, $Mg(NO_3)_2$, $NaNO_3$, $Al(NO_3)_3$, $Ba(NO_3)_2$, $Fe(NO_3)_3$, $Pb(NO_3)_2$, $Ce(NO_3)_4$, $Sr(NO_3)_2$, $LiNO_3$, $UO_2(NO_3)_2$, 0.1 gm. added per 208 gm. of soil; $Cd(NO_3)_2$, $Ni(NO_3)_2$, 0.02 gm. per 208 gm. of soil; and $Zn(NO_3)_2$ and HNO_3, 0.05 gm. per 208 gm. of soil. $Al(NO_3)_3$ and HNO_3 had no effect upon the number of nodules, but all other nitrates exerted a marked depressing result. Of the ammonium salts tested, the carbonate was without effect, the phosphate and aluminum ammonium sulphate were stimulating, while all others markedly decreased nodule production on soybeans.

ALBRECHT (1) found that the application of $NaNO_3$ corresponding to 10, 50 and 150 lb. of nitrate per acre did not appreciably decrease nodule formation by cowpeas, and that soybeans were not affected by amounts less than 150 lb. per acre.

GIÖBEL (8) has considered in some detail the effect of $NaNO_3$ upon soybeans and alfalfa. It was found that the gradual addition of the equivalent of 500 to 2600 lb. per acre of $NaNO_3$ to pot cultures of alfalfa was without result on nodule formation. When, however, the nitrate was all added at the beginning, 1200 to 1600 lb. per acre decreased the number of nodules to one fourth of normal. Amounts up to 1000 lb. of $NaNO_3$ were not effective. In another experiment soybeans were allowed to form nodules, and then $NaNO_3$ equivalent to 100 to 2100 lb. per acre was added gradually. The number of nodules was not consistently depressed, although the series containing about 300 lb. of $NaNO_3$ showed a decrease in weight of nodules. When the nitrate was added at the start, both the numbers and weights of nodules were decreased if more than 200 lb. of $NaNO_3$ per acre were applied. NH_4NO_3 was found to be twice as active as $NaNO_3$ in its effect upon the number of nodules. Generally, 100 to 200 lb. per acre of $NaNO_3$ greatly benefited the plant and stimulated nodule formation. GIÖBEL made nitrogen balances to determine whether nitrogen was fixed in the presence of the nitrate. He calculated the nitrogen fixed, by the difference between the nitrogen content of the tops of inoculated and uninoculated plants. Although the data show some fixation even when there was an excess of nitrogen added to the soil, GIÖBEL evidently did not consider this as significant, since he concludes: "Absorption of nitrates and fixation of atmospheric nitrogen by inoculated cultures took place simultaneously when the plants did not receive their full need of nitrogen from the soil. When this was the case, however, scarcely any fixation of nitrogen took place. In general the absorption of nitrates resulted in correspondingly smaller amounts of nitrogen being fixed. In other words the process of nitrate absorption dominated the process of nitrogen fixation." GIÖBEL's conclusions are probably sound, but the data offered are not without serious objections. In the first place he used an indirect method for estimation of nitrogen fixed, and in the second place, as he says, not all of the nitrate nitrogen that disappeared in his cultures could be accounted for in the nitrogen in the tops. This difference was ascribed to nitrate used for root development and *microbial assimilation.* In one experiment he added the nitrate in one-fifth portions at intervals distributed over the growth period and compared the results with those obtained with cultures in which all of the nitrate was added at the start.

He states that no differences could be observed in nodule counts or dry weight of plants because of this difference in treatment.

Ohkawara (10) grew lupine and serradella in small pots of sand. Different solutions containing 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 per cent. of nitrate were added in the proportion of 80 cc. of solution to 400 gm. of sand. The 0.2 and 0.5 per cent. KNO_3 solution and 0.5 per cent. $NaNO_3$ solution prevented the formation of nodules on the plants. 0.2 and 0.5 per cent. $Ca(NO_3)_2$ solution inhibited nodule production on serradella, and the 0.5 per cent. on the lupines. The solutions exhibited no effect or were slightly stimulating from 0.0 to 0.02 per cent., but all higher concentrations exerted a depressing action on nodules. $(NH_4)_2SO_4$ solutions in the same concentrations as the nitrates were also tested. The 0.05 per cent. solution decreased nodule formation, and the 0.1 per cent. solutions were found to be effective in preventing nodules.

In summary, the quantities of alkaline earth nitrates and ammonium salts which are required to affect nodule formation may be briefly presented as follows. In water cultures, concentrations of 1/4000 to 1/200,000 of nitrate nitrogen have been reported to prevent nodules from forming. Ammonium salts have been found effective in concentrations of 1/2000 to 1/100,000. For field experiments, 100 to 300 lb. of nitrate per acre depress or inhibit the formation of nodules, although some workers report that 1000 lb. are necessary. Small amounts of nitrate, however, appear to favor plant growth, and sometimes to increase the number of nodules.

Experimentation

Methods

The plant cultures were put up under aseptic conditions, and attempts were made to maintain sterility throughout the growth period. The plant used was mammoth red clover, which was set out in cotton-plugged 32-oz. round bottles containing the modified Crone's solution recommended by Bryan (2). In all cases, 0.8 per cent. agar had been added to the plant culture solutions. This concentration of agar gave a gel of proper rigidity, but which was still soft enough to offer very little resistance to the plant roots. The amount of nutrient agar used in each bottle was 200 cc. in experiment I, and 250 cc. in experiment II.

The nitrate used throughout the work was KNO_3. In some cases, varying amounts of this compound were added to the agar before sterilization; in other cases a small amount of nitrate was introduced at the start, and additions were made at intervals. The cotton plug of each bottle was pierced with an 8-mm. cotton-plugged glass tube. The nitrate was added through this tube as a sterile solution with a sterile 1-cc. pipette.

The methods employed in sterilizing and planting the seeds have been described previously by HOPKINS, WILSON, and FRED (9).

For inoculation, a suspension of a pure culture of *Rh. trifolii* strain number 209 was used; this organism has been found to increase greatly the total nitrogen content of the host plant. During the period of growth in the greenhouse the plant cultures were covered with layers of absorbent cotton. These caps were sprayed with a 5 per cent. solution of sodium benzoate containing a bacteriostatic dye. This method of preventing contamination has since been replaced by the use of 10-oz. waxed drinking cups inverted over the tops of the bottles.

The total nitrogen of the plants was determined by the Davisson-Parsons procedure (FRED and WAKSMAN, 7 p. 68) which includes nitrate-nitrogen. The nitrate remaining in the agar was estimated by a modification of this method. The agar was melted and made up to 500 or 1000 cc. After cooling, 50 cc. of approximately normal potassium alum solution was added with agitation. By filtering, a solution practically free of agar was obtained. The nitrate in a suitable aliquot of this was determined by alkaline reduction with Devarda alloy and distillation into $\frac{N}{28}H_2SO_4$. The filtrates of several cultures in each series were tested qualitatively for the presence of NH_3 N and NO_2 N but none was found.

EXPERIMENT I

SERIES 1.—Potassium nitrate was added to the agar at the start in varying amounts, 1, 2, and 4 mg. of NO_3 N per 200 cc. The same quantity of nitrate as originally introduced into the agar was added at intervals of 2 weeks for a period of 10 weeks. Thus, one set of 5 bottles contained 1 mg. of NO_3 N and 5 additions of 1 mg. each were made; a second set contained 2 mg. of NO_3 N and 2 mg. were added five times; a third set contained 4 mg. of NO_3 N and 4 mg. were added five times. The inoculated controls received no nitrate.

When harvested at the end of 4 months the plants of the three sets were very similar in size and color to the controls; all of the cultures were somewhat pale. Distribution and number of nodules were determined on all of the plants; the results are given in table I. In the column headed *No. of nodules* is given the mean of each treatment with its standard deviation.

SERIES 2.—4 mg. of NO_3 N were present in the agar at the start (except in the control), and nitrate was added at the rate of 1 mg. per week for varying lengths of time. In the first set, only one addition was made; in the second, two; and so on up to the last set which received nine additions of 1 mg. each. The control is the same as that used in series 1. The plants, which were 4 months old when harvested, resembled the controls; all

TABLE I

EFFECT OF ADDITION OF POTASSIUM NITRATE PERIODICALLY ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES PER 10 PLANTS	DIFFERENCE	PROBABILITY*	SIZE AND DISTRIBUTION OF NODULES
Control	8	124 ± 16	.	...	Principally on tap root, long type
Experiment I, series 1					
6 mg.	5	92 ± 5	32	0.15	Principally on tap root, both long and round
12 mg.	5	72 ± 8	52	0.04	Principally on secondary roots, mostly round and scattered
24 mg. . .	5	62 ± 12	62	0.02	Same as 12 mg. but smaller
Experiment I, series 2					
5 mg.	3	106 ± 7	18	0.50	Principally on secondary roots, mostly long type
6 mg. ...	4	96 ± 20	28	0.25	Same as 5 mg.
7 mg.	3	66 ± 20	58	0.07	Principally on secondary roots, mostly round and scattered
8 mg. . ..	3	61 ± 9	63	0.05	Same as 7 mg.
9 mg. ...	2	77 ± 18	47	0.20	Same as 7 mg.
10 mg.	3	83 ± 11	41	0.20	Same as 7 mg.
11 mg.	3	70 ± 15	54	0.09	Same as 7 mg.
12 mg.	2	91 ± 9	33	0.35	Same as 7 mg.
13 mg.	3	93 ± 27	31	0.30	Same as 7 mg.

Series I. 1 mg. at start; 1, 2, and 4 mg. respectively added every 2 weeks for 10 weeks.

Series II. 4 mg. at start; 1 mg. added per week until designated quantity reached.

* Probability that observed difference could have arisen from random sampling.

attained a fair size but were pale green. The results for series 2 also are given in table I.

EXPERIMENT II

SERIES 1.—The nitrate was added in varying amounts, about 3, 5, 10, 15, 20, and 40 mg. per bottle (250 cc.) at the start of the experiment, and no further addition was made during the growth period. The plants were harvested after seven weeks and examined for size and distribution of nodules as in experiment I. In addition, nitrogen determinations were made so that a nitrogen balance could be determined. In appearance, the high nitrate cultures were somewhat paler than either the low nitrate or the controls. The remainder of the data for this series are given in tables II and IV.

TABLE II

EFFECT OF ADDITION OF POTASSIUM NITRATE AT THE START ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES	DIFFERENCE	PROBABILITY	SIZE OF DISTRIBUTION OF NODULES
Control	11	73 ± 4		.	Principally on tap root, mostly long type
Experiment II, series 1					
3.3 mg.	4	70 ± 11	3	0.75	Principally on secondary roots, round and scattered
5.4 mg.	3	52 ± 10	21	0.04	Both tap and secondary Round and scattered
10.1 mg.	3	59 ± 10	14	0.15	Principally on tap roots Round and scattered
14.8 mg.	3	49 ± 10	24	0.02	Same as 10.1 mg. but slightly smaller
19.8 mg.	3	60 ± 9	13	0.20	Entirely on secondary roots, all round, small and scattered
39.0 mg.	3	36 ± 5	37	<0.01	Same as 19.8 mg.

SERIES 2.—Nitrate was added in the same amounts as in series 1 (3, 5, 10, 15, 10, and 40 mg.) except that its addition was extended over a period of 5 weeks. Three mg. of NO_3 N were added at the start to all cultures except the controls. The remaining nitrogen was added at weekly intervals; *e.g.*, to the 5 mg. set, 0.4 mg. per week; to the 10 mg. set, 1.4 mg. per week, and so on. The same controls were used for series 1, 2, and 3. The plants receiving nitrate were a paler green than the controls, the color and size of the plant tending to be adversely affected by the increase in nitrate. However, the differences were not so marked that they require consideration in interpreting the results. The plants were harvested after 7 weeks.

The data on the nodules are given in table III and those on nitrogen fixation in table IV.

TABLE III

EFFECT OF ADDITION OF POTASSIUM NITRATE PERIODICALLY ON NUMBER AND DISTRIBUTION OF NODULES

TOTAL NO_3 N ADDED	NO. OF SAMPLES	NO. OF NODULES	DIFFERENCE	PROBABILITY	SIZE OF DISTRIBUTION OF NODULES
Control	11	73 ± 4		..	Principally on tap root, mostly long type
Experiment II, series 2[1]					
3.3 mg.	5	54 ± 4	19	.01	Principally on secondary roots; both round and long, scattered
5.4 mg.	5	48 ± 8	25	<.01	Principally on secondary roots; round and scattered
10.1 mg.	5	43 ± 7	30	<.01	Same as 5.4 mg.
14.8 mg.	5	45 ± 8	28	<.01	Same as 5.4 mg. but smaller
19.8 mg.	5	22 ± 4	51	<.01	Same as 5.4 mg. but a few long type
39.0 mg.	5	27 ± 5	46	<.01	Same as 5.4 mg.
Experiment II, series 3[2]					
2.6 mg.	5	78 ± 12	5	0.60	Principally on secondary roots, both long and round, scattered
4.2 mg.	5	69 ± 12	4	0.70	Same as 2.6 mg.
7.4 mg.	5	65 ± 18	13	0.50	Principally on secondary roots, mostly round and scattered
12.2 mg.	5	54 ± 13	24	0.08	Same as 7.4 mg.
15.4 mg.	5	33 ± 3	45	<.01	Same as 7.4 mg.

[1] Series 2. 3.3 mg. NO_3 N at start. Remainder added in aliquots, weekly, for five weeks.

[2] Series 3. 2.6 mg. at start. Remainder added weekly at rate of 1.6 mg. per week.

SERIES 3.—It was intended that the additions of nitrate in this series should be in 2-mg. quantities as in series 1 and 2. However, on analysis of control cultures, *i.e.*, unplanted but receiving the same addition of NO_3 N it was found that the solution added each week contained 1.6 mg. NO_3 N instead of 2.0 mg. For this reason the total quantities of NO_3 N added in this series are somewhat less than the corresponding ones in series 1 and 2 of this experiment. In this series 2.6 mg. of NO_3 N were added to all the cultures at the start, then 1.6 mg. were added weekly until the following quantities were reached: 4.2, 7.4, 12.2, 15.4 mg. of NO_3 N. Thus only one addition was made to the 4.2 mg. set and eight additions were made to the 15.4 mg. set. At the time of harvesting (8 weeks) the plants

TABLE IV

EFFECT OF ADDITION OF POTASSIUM NITRATE UPON NITROGEN FIXATION

NO_3 N ADDED	NO. OF SAMPLES	NO_3 N IN AGAR AT END	NO_3 N USED	NITROGEN* IN SIX PLANTS	NITROGEN FIXED	PROBABILITY†
		mg.	*mg.*	*mg.*	*mg.*	
Control	11	0.0	0.0	4.1	4.06 ± 0.21	
Experiment II, series 1						
3.3	4	0.0	3.3	5.4	2.08 ± 0.55	0.04
5.4	3	0.2	5.2	6.9	1.70 ± 0.35	0.04
10.1	3	3.5	6.6	7.6	1.00 ± 0.34	0.10
14.8	3	8.3	6.5	7.5	1.00 ± 0.34	0.10
19.8	3	12.2	7.6	8.2	0.6 ± 0.53	0.35
39.0	3	31.9	7.1	6.5	−0.6 ± 0.60	0.45
Experiment II, series 2						
3.3	5	0.0	3.3	5.6	2.28 ± 0.16	<0.01
5.4	5	0.1	5.3	6.1	0.78 ± 0.25	0.04
10.1	5	3.5	6.6	7.5	0.94 ± 0.52	0.15
14.8	5	9.1	5.7	6.8	1.14 ± 0.51	0.10
19.8	5	13.8	6.0	6.2	0.20 ± 0.90	0.80
Experiment II, series 3						
2.6	5	0.0	2.6	5.8	3.20 ± 0.26	<0.01
4.2	5	0.0	4.2	6.5	2.28 ± 0.28	<0.01
7.4	5	0.4	7.0	8.0	1.06 ± 0.35	0.04
12.2	5	4.2	8.0	7.7	0.30 ± 0.20	0.20
15.4	4	9.2	6.2	7.1	0.90 ± 0.25	0.05

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* Corrected for uninoculated controls.

† Probability that observed fixation could have resulted from random sampling.

receiving the higher quantities of nitrogen (12.2 and 15.4 mg.) were paler in color than the remaining sets. The data are given in tables III and IV.

Discussion

The data given in the tables are best suited for discussion as a whole rather than individually; for this reason interpretation of the results of the various experiments has been reserved for this section of the paper.

EFFECT OF ADDED NITRATE ON NUMBER OF NODULES

In experiment I (table I) the number of nodules on the control plants, *i.e.*, those receiving no nitrate, was extremely variable; it will be noticed that the standard deviation of the mean is about 13 per cent. of the latter. Unpublished experiments in which we have made a study of the distribution curve of nodule formation in agar under the same conditions as were used in these experiments have indicated that the standard deviation of the mean should be about 5 per cent. of the mean. Because of the high variance in this experiment, significant decrease in the number of nodules in those plants receiving nitrate is rather difficult to demonstrate. The observed differences were tested by the "Student" method as modified by FISHER (3). Our experience has indicated that nodule formation satisfies the fundamental assumption of this method, *viz.*, that the estimated standard deviation is independent of the mean. The 4th column of the table gives the probability that the observed difference could have arisen from sampling. This probability is taken from a table which is entered by calculation of the statistic *t*:

$$t = \frac{X_1 - X_2}{s}\sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

$$s = \sqrt{\frac{\Sigma\,(X_1 - \overline{X}_1)^2 + \Sigma\,(X_2 - \overline{X}_2)^2}{n_1 + n_2 - 2}}$$

Where $\overline{X}_1$ is mean of n_1 observations (X_1), $\overline{X}_2$ is mean of n_2 observations (X_2); the table is entered at $n = n_1 + n_2 - 2$. A consideration of the formula shows that if the variance $\left[\frac{\Sigma\,(X_1 - \overline{X}_1)^2}{n_1}\right]$ of the controls is extremely high this will lead to large values of *s* independent of the variance in the treated samples. This large value of *s* will decrease the value of *t* which leads to a high probability that the difference arose from random sampling. For this reason it seems that any probability in this series of the order of 0.05 very likely indicates a significant decrease in the number of nodules. In series 1 the set in which 12 mg. were added at the rate of 2 mg. per week, and the set in which 24 mg. were added at the rate of 4 mg. per week, clearly decreased the number of nodules. In series 2, many of the treated samples have a high variance combined with a small number of samples; this leads to erratic results. Those samples that received 8 mg. of NO_3 N showed significant decreases in the number of nodules but the others did not.

In experiment II, the variance of the controls was much less and very similar to that found in previous experiments. On the other hand, the treated samples very often have extremely large values for the variance. This might be expected, since the effect of nitrate appears to be due to the disturbance of a delicate equilibrium of a nutritional character between plant and bacteria, and wide variations in results of the same treatment are not surprising. Thus in series 1 in which the nitrate is added at the start, it appears that the 5.4 mg., 14.8 mg., and 39.0 mg. sets show a decrease in the number of nodules whereas the 10.1 mg. and 19.8 mg. sets do not. On examination of the original data for these two sets, it was observed that two of the three samples taken showed a significant decrease in the number of nodules formed but the third sample was not affected. This led to a large variance and consequently an apparent high probability that the observed difference arose from sampling. It is likely with larger numbers of samples that this erratic response would be eliminated to a certain extent. In series 2 (table III) in which the nitrate was added over a period of 5 weeks, all the sets exhibited less variability in response to the treatment. In every case, even the 3.3 mg. treatment, there was a significant decrease in the number of nodules formed in the presence of the nitrate. In series 3, wide variation among the replicates in a given treatment was again noticed. For example, the number of nodules per six plants in this series were: 2.6 mg., 36, 101, 70, 101, 80; 4.2 mg., 116, 67, 49, 56, 58; 7.4 mg., 131, 50, 48, 45, 52; 12.2 mg., 101, 40, 59, 34, 38; 15.4 mg., 40, 35, 39, 28, 23. It will be noticed that in each set except the 15.4 mg., four of the five replicates checked very well in the response to nitrate addition but the 5th culture gave a large increase in the number of nodules which led to an increase in both mean and variance for the set. Consequently the effect on four of the cultures was obscured by the unusual behavior of a single culture. Calculation of the probabilities for this series, with the elimination of the erratic culture, gave: 2.6 mg., 0.09; 4.2 mg., 0.05; 7.4 mg., 12.2 mg., and 15.4 mg., < 0.01. Therefore it appears that in four of the cultures in all sets receiving more than 2.6 mg. of NO_3 N, addition of the nitrate decreased the number of nodules; the remaining culture showed an unexplained stimulation in nodule formation.

In general it seems that about 5 mg. of NO_3 N per 250 cc. is sufficient to cause a decrease in the nodule formation of clover plants grown in agar. There is also an indication that, as the quantity of NO_3 N is increased, there is a decrease in number of nodules formed, but this decrease is too variable to be of a linear nature. As far as could be determined, it made little difference in the response of the plant whether the addition of the nitrate was made at the start or periodically. Series 2, experiment II, indicated that addition of the nitrate over a 5 weeks' period was more effective than the other treatments, but this is without doubt partially due to the smaller variability in the data for this series as compared with the others. Finally it is to be noted that in none of our treatments was nodule formation entirely suppressed. The highest concentration used (39 mg. per 250 cc.) corresponds to a concentration of about 1.6 parts per 10,000. This exceeds the concentration that has been reported by various authors as sufficient to prevent formation of nodules. It is very likely that the quantity of nitrate nitrogen necessary for complete suppression of nodules on leguminous plants is variable, dependent on the growth of the plant, which in turn is dependent largely on experimental conditions used.

EFFECT OF ADDED NITRATE ON THE SIZE AND DISTRIBUTION OF THE NODULES

The use of the number of nodules as a criterion for evidence of suppression of the activity of rhizobia in leguminous plants is not entirely satisfactory. It was shown in these experiments that the variability in the response of the plants as measured by the number of nodules often obscures the interpretation of a treatment. Because of erratic individuals in a set, differences that are significant are not always apparent when a statistical test is used. Other workers encountering this have discarded the nodule count for other methods. Thus GIÖBEL used both fresh and dry weight of nodules as a measure of the response of soybeans to the addition of nitrates. He found that in many cases no significant decrease in the numbers of nodules could be discerned but that the weight of nodules was inversely proportional to the quantity of nitrate nitrogen added. Furthermore, there appeared to be a sharp correlation between mass of nodules and nitrogen fixed. While in our experiments it was not possible to weigh the nodules, observations on the size and distribution were made. As shown in tables I, II, and III, the effects of nitrate on size and distribution are extremely consistent. The nodules on the controls were located principally on the tap root, often near the crown; the majority of them were of the long finger-like type. The addition of small amounts of NO_3 N altered this. More of the nodules were found on the secondary roots and the round type of nodule was in evidence. As the quantity of NO_3 N added was increased, the nodules were found almost exclusively on the secondary roots and became progressively smaller and more scattered. This effect on size and distribution of nodules appeared to be fairly independent of the method used for adding the nitrate. However, it does appear that about

3 mg. per 250 cc. at the start is necessary to cause the distribution to change from the tap root to the secondary roots, since in series 1, experiment I, many of the nodules in the 6-mg. set were of the long type and on the tap root. In this set there was only 1 mg. of NO_3 N at the start and 1 mg. was added weekly. In the other series at least 3 mg. were present at the start and in all cases both the 3- and 5-mg. set showed the nodules distributed principally on the secondary roots. From these data it is apparent that observation of the size and distribution of the nodule is a decidedly more consistent method of measuring the effect of nitrate on inoculated plants than is the counting method, even though the results can be expressed only in a qualitative manner.

EFFECT OF ADDED NITRATE ON NITROGEN FIXATION BY THE PLANTS

The nitrogen balance for experiment II is given in table IV. These data show that even the smallest quantity of NO_3 N added was effective in decreasing the amount of nitrogen fixed by the association of organism and plant. However, in the presence of nitrate the total quantity of nitrogen found in the plant was increased so that a slight fixation did occur. For example, fixation took place in the 5-mg. set in every series, although it appeared from the controls that about 4.0 mg. were sufficient to meet the needs of the plants grown under these conditions. This suggests that the presence of the nitrate stimulates the plant to a certain extent and a better growth results. It is probable that this stimulation is due to the beneficial effect on the plant of a source of nitrogen during the period of nitrogen starvation, *i.e.*, before nitrogen fixation begins. Finally, it should be noted that in every case in which there is present an excess of nitrate there was no significant fixation. In the border line cases, *viz.*, about 10 mg., there often appears to be a slight gain (order of 1 mg.), but when the errors attendant on the nitrate determination in the agar are taken into consideration, this fixation cannot be considered significant. The results for the 15.4-mg. set in series 3 appear to indicate a slight fixation in this set. This might have been because the small quantity added each week (1.6 mg.) was insufficient for the needs of the plant in the early stages. However, since no fixation was observed in the 12.2-mg. set in this series, it is more likely that apparent fixation was obtained because of errors in analysis of the nitrate left in the agar.

From these data it is concluded that the addition of NO_3 N to inoculated plants does not prevent nodule formation by the bacteria but it does delay establishment of the bacteria in the host plant and modify their behavior toward the latter. Even the addition of quantities of NO_3 N that are much in excess of the requirements of the plants does not entirely prevent the formation of nodules; however, these nodules do not develop normally and appear to act parasitically on the plant. A very delicate equilibrium must exist between the needs of the plants for nitrogen and the fixation process; in all cultures which were not supplied with sufficient nitrogen to meet the needs of the plant, fixation occurred, but as soon as an excess of nitrogen was added, the fixation process appeared to be entirely suppressed in spite of the fact that nodules were present. These effects were independent of whether the nitrate was added at the start or periodically to the plant culture. In the latter case, the addition of NO_3 N at the rate of 2 mg. per week was evidently sufficient to keep a supply available to the plant, if this addition was continued throughout the growth period.

Summary

1. The addition of nitrate nitrogen to clover plants grown in agar generally resulted in a decrease in the number of nodules formed when the concentration exceeded 2 to 3 parts per 100,000. However, the response of the plant to nitrate addition was erratic, and significant decreases were not always noted. Whenever decreases were observed, the effect appeared to be independent of whether the nitrate was added at the start or periodically. However, the most consistent depression of nodule formation was noted in the series in which the nitrate was added over a period of 5 weeks. Complete prevention of nodule formation was not effected by even the highest concentration of NO_3 N used, *viz.*, 1.6 parts per 10,000.

2. The distribution and size of the nodules were markedly affected by all the concentrations used. Addition of even small quantities of nitrate brought about formation of scattered nodules, largely of the round type, on the secondary roots; whereas normally the nodules are mostly of the long type and are found on the tap root near the crown. The size of the nodules was decreased as the quantity of nitrate was increased. These results were independent of the method of adding the nitrate provided a certain minimum quantity of nitrogen was available at the start.

3. All concentrations of nitrate nitrogen used markedly decreased the fixation of free nitrogen. If the quantities of NO_3 N added were insufficient for the needs of the plants, the deficit was made up by fixation of atmospheric nitrogen. However, as soon as an excess of nitrogen was provided, the fixation process appeared to be completely suppressed, even though nodules were present.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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INFLUENCE OF SOME ENVIRONMENTAL CONDITIONS ON THE GROWTH OF EXCISED ROOT TIPS OF WHEAT SEEDLINGS IN LIQUID MEDIA

PHILIP R. WHITE

(WITH FOUR FIGURES)

Introduction

The experiments reported in this paper are of a preliminary nature, dealing with what may reasonably be expected from solution cultures of excised roots of wheat seedlings. In a certain sense (**11**) the culture of excised root tips may be considered as lying within the field of tissue culture, a field that has received much attention at the hands of animal physiologists and one that clearly deserves much more attention among plant physiologists than it has received thus far.

Materials and methods

Use of liquid media. The most satisfactory experiments on the culture of excised root tips thus far reported appear to be those of ROBBINS (**4, 5**) and ROBBINS and MANEVAL (**6, 7**), and the technique of the present study was largely based on the methods employed by these writers. Liquid media were used. Some authors, for example DIETRICH (**1**), KOTTE (**2**), and ÚLEHLA (**9**), have regarded liquid media as unsuited to such experiments as those here considered; but it is obvious that the use of liquid media avoids many complexities that are inevitable when water-impregnated solid media are employed. It is surely desirable, in approaching any new line of experimentation, to begin with the simpler kinds of systems, and the solution-culture method is relatively simple, with regard to the preparation of more or less known media as well as to the analytical interpretation of results. It is of course clear, although not always brought into biological discussion, that the indications of any experiment are directly applicable only to the set of conditions and circumstances under which the experiment was performed, and undue generalizations are to be avoided. The study here reported dealt only with the growth of these excised root tips in aqueous solutions. How similar root tips might behave in other media is of course a subject for direct experimentation.

Wheat seedlings were chosen as the source of experimental material since the physiology of the wheat plant is of special interest because of its great agronomic importance in many parts of the world, because wheat has already been studied in many ways, and because wheat seedlings present relatively few difficulties of manipulation in such experimentation as is dealt with here.

The distilled water used in the nutrient solutions was finally prepared by triple distillation of spring water, first over alkaline potassium permanganate, then over barium hydroxide, and finally without any addition. The whole process was carried out in "Pyrex chemically resistant" glass and the water was stored in containers of the same material. This gave a very uniform product, of very low electric conductivity (about 3.4×10^{-6} reciprocal ohms), and without any apparent toxicity. Even with very nearly this same low conductivity, toxicity was evident in many instances when water distilled in other ways was tried.

The chemicals used were of high grade and no attempt was made to purify them or to take into account such traces of impurity as were present according to the makers' labels. The culture vessels were 125–ml. Erlenmeyer flasks, of "Pyrex chemically resistant" glass. Each was supplied with 50 ml. of medium, with a single root tip. Flasks were used repeatedly, always being cleaned by thorough rinsing, immersing in cleaning fluid (sulphuric acid and potassium bichromate), rinsing first in running hot water and then three times in ordinary distilled water and finally once in triple-distilled water, after which they were allowed to drain before charging.

The root tips used were taken from seedlings produced under standard conditions. A lot of seed supplied by a commercial firm showed a high proportion of defective seeds and much tardy germination, and most of this study was carried out with a supply of a "pure-line" Marquis wheat (C. I. no. 3641), kindly supplied by the United States Bureau of Plant Industry. The seeds used were selected by hand and eye with respect to size and general appearance, all that appeared unusual being discarded. These seeds were soaked in WILSON's (**12**) calcium-hypochlorite sterilizing solution for 3–5 hours, in a bottle continually rotated at a speed of about 60 revolutions per minute. The treated seeds were thoroughly rinsed in heat-sterilized distilled water, after which they were distributed in heat-sterilized petri dishes (10 cm. in diameter), 12 seeds in each dish. On the bottom of each dish, beneath the seeds, were two sheets of filter paper (Whatman, no. 1) moistened with about 3 ml. of heat-sterilized distilled water. The seeds were evenly spaced on the paper circles in order to permit roots to develop without too many contacts. After the covers had been placed on them the dishes were set aside in a dark room with the temperature artificially maintained at about 25° C., and germination was allowed to proceed for about four days, until the first roots had attained a length of about 2 cm.

From the supply of apparently normal roots thus obtained, terminal 3-mm. tips were removed by means of a sharp scalpel, each tip being promptly introduced into its culture flask, which had previously been charged with the requisite solution. The excised portion included all of the meristematic region (which is translucent in these roots) and also about 1 mm. of the region of elongation (which is more opaque and more nearly white in appearance). These manipulations were performed with precautions designed to avoid the entrance of bacteria and fungus spores into the flasks. Serious troubles due to microörganisms were encountered in some instances, but obviously contaminated cultures were discarded. It was noted that tips might show injury, as if by traumatic shock, if the scalpel used had not been very sharp or if they had been handled carelessly. Injured tips failed to elongate promptly, although in many instances they appeared to have recovered after a day or two and then began to lengthen.

Excepting some cultures in very unfavorable media (such as distilled water), well prepared tips showed no initial retardation of elongation, and their length was about doubled in the first 24 hours after excision. In most of the solutions tested they maintained this growth rate for three or four days, after which the rate of elongation was apparently controlled by the nature of the surrounding solution. The initial period during which the characteristics of the medium generally failed to produce any notable effect on the rate of growth may perhaps represent the time required for the supply of some materials originally present in the tissues to become exhausted, but the delicate and difficult problem thus suggested was not attacked.

The basic mineral-nutrient solution contained the following six inorganic salts, with the concentrations given (in milligrams per liter): $Ca(NO_3)_2 \cdot 4H_2O$, 144.0 mg.; $MgSO_4 \cdot 7H_2O$, 50.0 mg.; K_2CO_3, 34.8 mg.; KNO_3, 25.0 mg.; KH_2PO_4, 25.0 mg.; $Fe_2(SO_4)_3$, 1.25 mg. This formula was used by USPENSKI and USPENSKAJA (**10**) in their studies on *Volvox.* It contains all of the ions that are generally essential, in considerable concentrations, for plant growth. By means of preliminary tests it was chosen from among seven more or less different solutions used for plant cultures by various experimenters, mainly because it was found to maintain itself without opalescence or precipitation throughout a wide range of H-ion concentration (from pH 5.0 to pH 9.0), while the others all failed in this respect. That the basic solution should show no precipitation throughout such a range was essential in the present study because it was planned to devote some attention to the influence of H-ion concentration on root-tip growth and many pH values would need to be tested. The USPENSKI solution is unusually dilute; its total salt concentration is less than one-seventh that of the KNOP-PALLADIN solution (**3**); and it is only about one thirty-sixth as concentrated as the SHIVE (8) 3-salt solution (R_5C_2, osmotic value of 1.75 atm.). As the experimental results showed, the salts, salt proportions, and total concentration of the USPENSKI solution proved to be very suitable for the cultures here considered.

Dextrose and yeast extract were regularly added to the basic solution just described, since ROBBINS (5) had found them favorable to the growth of excised root tips. Dextrose was added in the proportion of 20 gm. per liter of solution and yeast extract in such amounts as to give a final concentration of an extract of 100 mg. of yeast per liter. The extract was prepared by boiling 0.5 gm. of dried brewers' yeast (obtained from Wallerstein, New York City) for half an hour in 100 ml. of distilled water. This was then filtered and allowed to cool, after which the filtrate was made up to the original volume. Twenty ml. of this extract added to a liter of the solution gave the required concentration. A fresh supply of yeast extract was prepared for each series of cultures. In all series the solutions were distributed immediately to their respective flasks, which were autoclaved for 20 minutes at a pressure of 1.25 atm. (20 lb. per sq. in.) and then set aside for 1–4 days before inoculation.

The general experimental conditions and procedures for all cultures of a series were nearly uniform. Four hundred simultaneous cultures constituted each series. The 400 flasks stood in 40 rows of 10 each, on a long table along the wall of a well ventilated basement room with maintained temperature of about 25°. Continuous electric illumination was regularly supplied by means of Mazda nitrogen-filled "daylight" lamps. Except in the experiments on H-ion concentration as such, no attempt was made to control the acidity of the media, but in almost all series the pH value of each modification of the culture solution was recorded, using a Youden quinhydrone-electrode potentiometer. Within each series a number of different conditional complexes were tested and each test was regularly repeated at least once. Results were accepted as valid only when two or more tests were in good agreement. The experimental period was two weeks in length. Throughout this period the culture flasks were kept closed with cotton plugs. The solutions were not renewed. Measurements of root length (in millimeters) were made only at the end of the experimental period. At that time the root tips were removed from the culture flasks and spread out under water to facilitate examination. For each culture the length of the main root, the length of all branch roots taken together, the number of branch roots, and the total length of main root and branch roots combined were recorded, together with notes on general appearance. For each set of conditions the mean of the total-length values of all roots grown under that conditional complex is taken as a numerical index of growth.

Experimental results

Water, mineral salts, dextrose, and yeast extract made up the full nutrient medium for these cultures, as has been said. With suitable temperature and illumination (see later) this gave a growth index of 121.1 mm. A representative root tip, grown 2 weeks in this medium at 25° and with artificial illumination, is shown at *A* in figure 1. Elongation and branching continued throughout the period and the root tips appeared healthy and vigorous at the end.

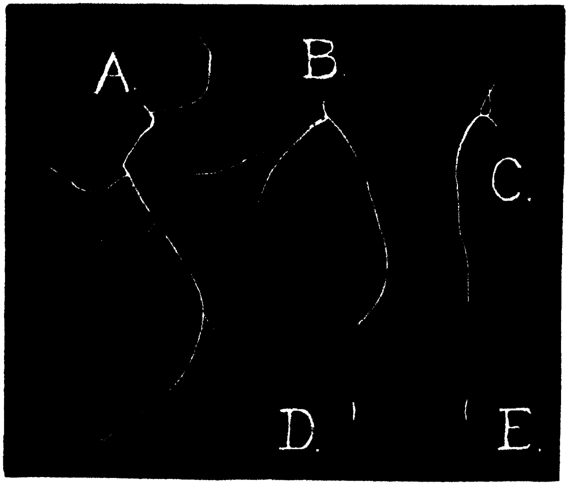

FIG. 1. Representative root tips grown two weeks at 25° and with continuous electric light, approximately natural size: *A*, cultures in full nutrient medium (redistilled water to which salts, dextrose, and yeast extract had been added); *B*, cultures in similar medium but without yeast extract; *C*, cultures in medium same as for *A* but without the salts. The two small roots shown at the bottom, without significant growth, represent cultures with a simple dextrose solution (*D*) and with redistilled water to which only yeast extract had been added (*E*).

An otherwise similar medium from which yeast extract had been omitted gave a corresponding growth index of only 72.0 mm. A representative root tip from this modified solution appears at *B* in figure 1. While growth here was somewhat marked and generally continued throughout the period, it was much less rapid than when salts, dextrose, and yeast extract were all supplied. Omission of yeast extract apparently reduced the growth index by about 40 per cent. in the 2-week experimental period; nevertheless the root tips appeared healthy at the end of the period.

Omission of the mineral salts from the full nutrient medium gave a still smaller growth index, only 42.3 mm., and permitted little or no branching.

Of course the solution thus modified supplied both dextrose and yeast extract, and its osmotic value was closely similar to that of the regular medium. A representative root tip from this solution is shown at *C* in figure 1. Much of the growth occurred in the early part of the experimental period and elongation soon became very slow or ceased altogether. Variability in extent and manner of growth was greater with this medium than with either of the two media just referred to, and the appearance of the root tips at the end of the experiment suggested poor health.

Distilled water with dextrose alone or with yeast extract alone gave only insignificant growth or none at all, and such slight elongation as did occur in these media was confined to the very early part of the experimental period. Nevertheless the root tips appeared to be still alive at the end of the period. The two small root tips (*D* and *E*) at the bottom of figure 1 are representative samples from these media. The difference in shape is not significant. The osmotic value of the simple solution of dextrose was of course nearly like that of the full nutrient medium, while distilled water with yeast extract alone had a much lower osmotic value.

Distilled water alone was apparently neither better nor worse for these root tips than was distilled water with addition of dextrose or of yeast extract. Some tests with tap water without any addition gave results like those obtained with distilled water either alone or with addition of dextrose or yeast extract.

It appears that neither dextrose alone nor yeast extract alone (with its uncertain but surely small content of mineral salts and organic material) could supply the requirements for growth of these excised tips, even for a short time. Addition of dextrose or of yeast extract to distilled water did not overcome the inadequacy of the water, although the food value of dextrose cannot be questioned and although the dextrose solution had almost the same osmotic value as the full nutrient medium, which gave pronounced growth. It may be supposed that dextrose not only supplied the carbohydrate needed for cell formation and extension but also furnished a requisite source of energy through respiration, and that inorganic nutrition was at first possible through salts originally present in the root tips. Some inorganic and also some organic material was doubtless derived from the yeast extract. Apparently the internal mineral supply and the salts derived from yeast extract were not adequate to maintain growth beyond the first few days. That rather good growth was obtained when the salts of the USPENSKI formula and dextrose were both externally supplied indicates, as might be expected, that these root tips required, for maintained growth, a continuous external supply of both carbohydrate and salts. When both dextrose and salts were present in the medium, growth was greatly accelerated by yeast extract, presumably because of the presence of some very active material derived from yeast or made available through the action of something derived from yeast.

That growth in the first two weeks of culture was nearly doubled when yeast extract was added to an already good solution is of special interest. It will be recalled that this effect was produced by extract from only 0.1 gm. of dry yeast per liter of solution. ROBBINS (5) tested a similar yeast extract and concluded that it was *not* beneficial to the growth of his maize root tips in the first two weeks after excision. However, he observed a stimulative effect due to his yeast extract, but that effect was manifest only after about six weeks of culture. The experiments of ROBBINS are of course not directly comparable with those reported here, for he was studying maize and he employed much larger pieces of root (mostly 10–60 mm. long) than were used in the present study.

Relations between growth and H-ion concentration of the medium received some attention. A series of cultures included sixteen different solutions, each represented by eight different initial pH values, as follows: 5.0, 6.0, 6.4, 6.8, 7.2, 7.6, 8.0, and 9.0. Adjustments of H-ion concentration were made with 0.01 M HCl or 0.2 M NaOH and pH values were measured with a potentiometer and Youden quinhydrone electrode, also being verified occasionally by use of the glass electrode. No attempt was made to buffer the solutions, since phosphate or borate buffers would have altered the concentrations of the constituent inorganic ions while citrate, phthalate, or glycine buffers would not only have affected the ion concentrations but would also have added organic constituents which might have influenced growth. This consideration, which seems to have been largely ignored in many studies on H-ion concentration, requires attention because it is difficult or impossible to distinguish between buffer action itself and other effects that might possibly be related to the buffer substances.

There were three cultures for each initial pH value of each solution and, in addition, a single blank flask (without root tip) for each solution as it was before adjustment. All flasks and their contents were autoclaved initially, but no H-ion measurements were made after autoclaving until the end of the culture period, after the root tips had been removed from the culture flasks. Consequently no information is available concerning the actual pH values when the roots were introduced into the solutions, and it is possible that hydrolysis, or other alteration of the solutions brought about by autoclaving, may have been somewhat different for originally different pH values of the same solution, and that pH values when the root tips were introduced may have been somewhat different from the corresponding values before autoclaving.

In general there was much apparently irregular variability in this series and most of the results may be omitted here, but three solution types are considered briefly. They are designated as A, B, and C. These differed only with respect to salt content and salt proportions. All three were alike in that they contained per liter 20 gm. of dextrose and the extract from 0.1 gm. of yeast.

Solution A was like the full nutrient medium except that KH_2PO_4 (25 mg. per liter) was replaced by KCl (14 mg. per liter). The final pH values for the eight different initial H-ion concentrations of this solution were all nearly alike, and low (about 5.1), although the initial values (before autoclaving) ranged from 5.0 to 9.0, as has been noted. Autoclaving and standing for two weeks in contact with a root resulted, therefore, in altering the initial pH value only a little if it was low, but producing increasingly great reduction as the initial value was higher. An initial value of 9.0 became 5.3 at the end of the experiment. Such alteration might be expected, for this solution contained no phosphate (unless a trace of PO_4-ion may have been introduced as impurities or in the yeast extract) and the salts used have but little buffer action. The growth indices varied irregularly and no consistent relation was apparent between growth of the root tips and either initial or final pH values. Nevertheless all but the initially most alkaline variant of this solution ($pH = 9.0$) gave good growth.

Solution B was the full nutrient medium already referred to. As in the case of solution A, the final pH values were all low but they were consistently somewhat higher as the initial value (before autoclaving) was higher. For the initial series of values specified, from 5.0 to 9.0, the final values ranged in an almost linear series from 5.0 to 5.65. The differences in H-ion concentration were thus maintained in their directions throughout the 2-week period, although their magnitudes were generally very greatly decreased. The pH value that was initially lowest did not alter, but all the other initial values became very much lowered, more so as their magnitudes were greater. In this connection it is to be noted that solution B contained a considerable concentration of phosphate, an effective buffer, which was absent or present in only exceedingly low concentrations in solution A. The growth indices for the eight different initial pH values of solution B showed irregular variations, but these were not so pronounced as in the former instance. The lowest index value (41 mm.) was for the culture whose solution had the highest initial and final pH value, but all the other growth indices were without consistent relation to the pH value.

Solution C differed from solutions A and B (the full nutrient medium) only with regard to its phosphate concentration; it contained twice as much KH_2PO_4 per liter as is called for by the USPENSKI formula, and it was consequently more effectively buffered. Its final pH values were regularly lower than the corresponding initial values, as in the cases of solutions

A and B, the lowering being more pronounced as the initial values were higher. For initial values from 5.0 to 7.2 inclusive the final values formed a nearly linear ascending series from 4.8 to 5.9, but for initial values from 7.2 to 9.0 inclusive the final values were sensibly alike, about 5.95. Although there was considerable irregular variation in growth-index values, a rather consistent relation between growth and H-ion concentration is suggested by the numerical data for this series, which are as follows:

Initial pH values	5.0	6.0	6.4	6.8	7.2	7.6	8.0	9.0
Final pH values	4.8	5.0	5.3	5.4	6.0	5.6	6.0	5.95
Final growth-index values (mm.)	140.0	153.3	119.3	144.3	67.7	47.3	49.3	35.0

The growth index may be taken as about the same (from 119.3 to 153.3 mm.) for final pH values from 4.8 to 5.4, while it tended to be progressively somewhat smaller for initial pH values from 7.2 to 9.0. As has been said, the initial pH values refer to the solutions before they were autoclaved, and no information is at hand to show whether either the initial or final pH values really represent the environments of the root tips, or whether the initial or the final pH values were more effective during a larger part of the experimental period, etc. It is consequently not desirable to attempt any closer analysis of these relationships. An equilibrium value about 5.0–5.5 may be regarded as optimal for growth of these wheat root tips in the full nutrient solution (Uspenski solution plus dextrose and yeast extract) and under the other conditions of this study. This represents a much greater acidity than has usually been maintained for cultures of animal tissues.

The three control solutions that were left unadjusted as to H-ion concentration and stood for two weeks without root tips showed final pH values of 5.1 (solution A), 4.8 (solution B), and 5.2 (solution C). In another experiment five flasks of a solution containing the usual proportions of salts and dextrose but no yeast extract were allowed to stand two weeks without root tips. They gave a mean final pH value of 5.55 ± 0.25. Robbins found that his culture solutions generally showed a final pH value about 5.4, and suggested that H-ion adjustment during the experimental period was brought about through some sort of buffer action due to root tips or yeast extract. This was obviously not the case in the control tests of the present study. Robbins does not give final pH values for any solutions that were allowed to stand throughout the experimental period without root tips or without root tips and yeast extract.

The influence of temperature on growth of these root tips of wheat seedlings in the full nutrient medium was studied by means of two experimental series, both conducted in darkness. In the first of these, six different, maintained temperatures (15°, 20°, 25°, 30°, 35°, 40°) were tested. There were nine cultures for each temperature. At the end of the 2-week period a marked influence of temperature was indicated, as is shown by the representative roots depicted in figure 2. For 15° the growth index was 15 mm.; the tips appeared healthy but had very few and very short branches. For 20° the condition of the roots was about the same as for 15° but the growth index was 23 mm. For 25° the condition was excellent and the growth index was 62.5 mm., almost three times as great as for 20°; on the average there were seven branches per root tip. For 30°, on the other hand, the roots were evidently unhealthy; the growth index was only 8.2 mm. and there was only one branch per root tip on the average. For 35° and 40° the tips were blackened and swollen; no branches were present and no elongation had occurred. The optimal temperature appears to have been between 20° and 30°.

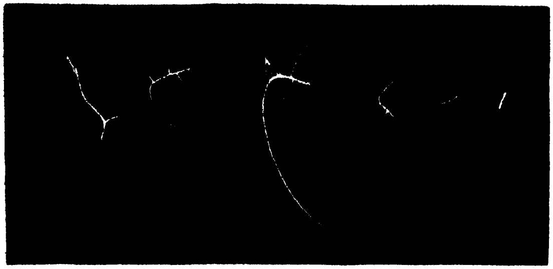

FIG. 2. Representative excised root tips grown two weeks in full nutrient medium (salts, dextrose, and yeast extract in redistilled water) and in darkness, at six different maintained temperatures. About natural size. Temperatures represented are, reading from left to right: 15°, 20°, 25°, 30°, 35°, and 40°.

The second temperature experiment included maintained temperatures of 20°, 22°, 24°, 25°, 26°, 27°, 28°, and 30°, for which the growth indices

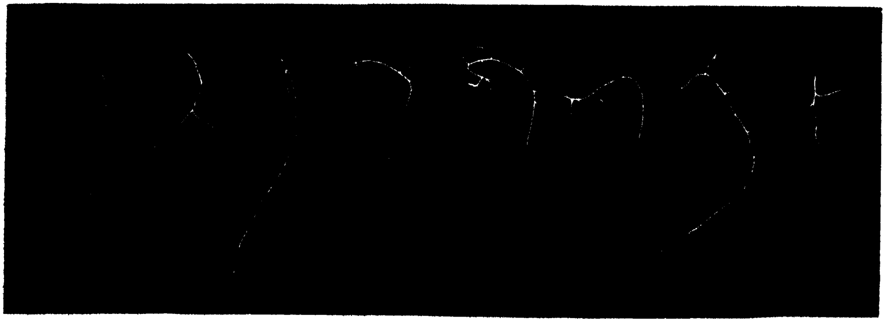

FIG. 3. Same as figure 2, but the maintained temperatures represented are, reading from left to right: 20°, 22°, 24°, 25°, 26°, 27°, 28°, and 30°. About ¾ natural size.

were respectively 81, 98, 89, 75, 74, 59, 91, and 33 mm. Representative root tips from this series are shown in figure 3. The highest temperature was obviously supra-optimal but no optimal temperature is clearly indicated. Nevertheless the appearance of the root tips and the manner of their growth indicated that the optimal temperature was about 26°–28°.

The influence of illumination on the growth of their excised maize root tips was considered briefly by ROBBINS and MANEVAL (7), who concluded that light was beneficial; but it was not clearly shown that temperature differences between lighted and unlighted flasks may not have been more or less effective in their experimental tests. To distinguish between light effects and those of heat is very difficult indeed. In the present study of excised wheat root tips some comparatively simple experiments were devoted to the influence of illumination on growth. For cultures in darkness each flask was covered with an opaque paper wrapper, black within but white on the outside. The cultures were distributed uniformly on a table, each row of covered flasks being accompanied by an adjacent row of uncovered ones.

One experiment comprised sixteen different solutions, with ten darkened and ten lighted cultures for each solution. The light supplied was ordinary diffuse daylight. All cultures were in darkness throughout the nocturnal part of each day, while the root tips in uncovered flasks received natural and fluctuating weak light during the diurnal part of each day. Those in covered flasks were in darkness throughout the entire 2 weeks of the experiment. It was presumed that all cultures had nearly the same temperature, about 22°. Cultures with solutions that were well suited to the growth of these root tips showed significantly more growth in the uncovered flasks than in the covered ones. With solutions that were not well suited to growth, however, this apparent influence of light failed to appear or was so slight as to be insignificant.

Another experiment on the influence of illumination comprised one hundred cultures in the full nutrient medium, fifty in covered flasks, and fifty in uncovered ones. The flasks stood on a table in a room provided with fair temperature control (about 25°) and with continuous electric light. Three 150-watt nitrogen-filled Mazda "daylight" bulbs with reflectors furnished illumination; they were 70 cm. above the plane of the table, about 75 cm. apart, and in a straight row, being so placed as to illumine the entire series about equally. Three covered and three uncovered flasks, each of which bore a thermometer whose bulb was immersed in a volume of water equal to the volume of solution regularly used, gave evidence concerning the culture temperatures. These were arranged in pairs at the left, center, and right of the group of cultures. The approximate mean temperature of the water in the uncovered thermometer flasks for the 2-week period was 25.0°, while the corresponding mean temperature of the water in the covered ones was 0.7° lower. If it be supposed that these temperature values properly represent the temperature of the solutions in the two sets of culture flasks, it may be said that the illuminated root tips were kept generally about 0.7° warmer than those without light.

The average growth index for the fifty darkened cultures was 103.0 mm. while the corresponding value for the fifty illuminated (and presumably somewhat warmer) cultures was 120.2 mm. This is surely a significant difference; it amounts to 16.5 per cent. on the basis of the lower value. Furthermore, a similar difference was clearly indicated by ocular evidence; the darkened root tips were more slender and more sparsely branched than the others and they appeared less healthy; those grown with illumination were less slender, more richly branched, and apparently in better general condition, although somewhat more bent. In figure 4 a representative sample of the dark-grown roots is shown at the left and a representative sample of the others is shown at the right.

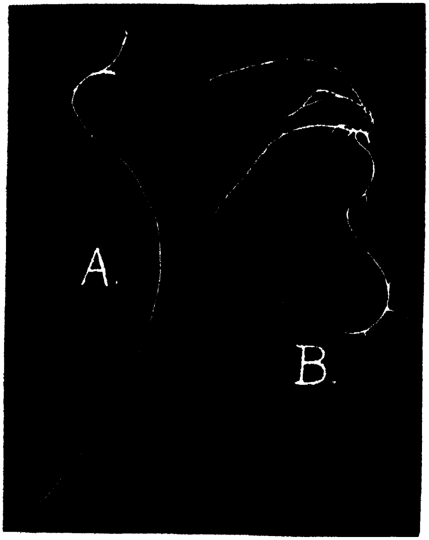

FIG. 4. Representative excised root tips, approximately natural size, grown two weeks in full nutrient medium: *A*, in darkness at about 24.3°; *B*, with continuous electric illumination, presumably at a temperature a little higher than that of the cultures in darkness.

Although a temperature difference of the order of 0.7°, maintained for two weeks, is not by any means to be regarded as negligible, yet it may be recalled that the experiments on temperature influence (see preceding discussion) gave no indication of great sensitiveness in these root tips to slight differences in temperature in the region of 24°–26°, and it consequently seems unlikely that the pronounced growth difference was mainly due to unlike temperatures of the darkened and illuminated solutions. It may therefore be said that these cultures of excised wheat roots showed a marked beneficial or stimulating effect due to such illumination as was tested. This conclusion supports the indications derived from the preceding experiment on illumination. ROBBINS and MANEVAL (6) came to a similar conclusion with regard to their excised maize root tips. They reported also that anthocyan was formed in their illuminated maize roots, but no coloring was observed in the cultures here described.

The degree of aeration of the culture solution might exert an influence on the rate of growth of these wheat root tips if the external supply of oxygen were a limiting condition. The general question of aeration was discussed to some extent by ROBBINS and MANEVAL (7).

Some experiments on aeration were carried out in the present study and their results remain to be presented. No elaborate procedures for securing different degrees of aeration of the medium were attempted, partly because it seems best to begin to approach new problems by means of relatively simple methods and partly because time and facilities for adequate control of oxygen supply and the disposal of CO_2, etc., were not readily available. The experiments of ZIMMERMANN (13) gave rise to the suggestion that depth of culture solution might serve as a rough control of oxygen concentration in the immediate vicinity of the root tip, and several different depths of solution were accordingly tested. The flasks used were all of the same size, but some had 50 ml. of solution, others had 25 ml., and still others had 10 ml. The three resulting depths of solution were approximately 20, 10, and 4 mm. respectively. All three groups exposed about the same area of solution to the air above (approximately 28 sq. cm.). Test-tubes containing 10 ml. of solution were also used, with liquid columns about 6 cm. high and an air-liquid area of about 2.5 sq. cm. Because the root tips generally lay at the bottom of the flasks, beneath the solution,[1] all oxygen reaching them from the air had to penetrate the solution either by solute diffusion or by convection, or by both means. To secure in this way different depths of solution in flasks of the same size naturally involved the introduction of another experimental variable, namely, the volume of medium present. But the test-tube and the flask containing the least volume of medium were comparable with respect to depth and area, for the volume of solution used was 10 ml. in both instances. For these aeration tests the full nutrient medium was used; that is, the Uspenski solution plus dextrose and yeast extract. Flasks and test-tubes stood in a room with temperature fluctuating approximately between 21° and 23°, with continuous artificial illumination provided by five 150-watt Mazda "daylight" lamps arranged as in the illumination tests already described.

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[1] This was not always true. Although the root tips regularly sank to the bottom of the flasks immediately upon being introduced, yet in many instances rapidly growing tips subsequently rose to the surface and remained there. No difference was observed, in either manner or rate of growth, between sunken and floating root tips. This constitutes indirect evidence that aeration was adequate, even at the bottom of the flasks.

The average growth indices for the four groups of containers, ascertained at the end of the 2-week experimental period, were as follows:

Flasks:	Greatest depth,	50 ml. of sol.,	44.2 mm.
	Medium depth,	25 ml. of sol.,	41.3 mm.
	Least depth,	10 ml. of sol.,	32.3 mm.
Test-tubes:		10 ml. of sol.,	32.8 mm.

Aeration of the root tips was apparently adequate in both the flasks containing the medium volume of solution and in those containing the largest volume. This seems to imply that oxygen supply was more than adequate in the latter, which had the same volume as those regularly used throughout this study. The significantly smaller growth indices for the flasks that contained the smallest amount of solution is clearly not to be considered as due to excessive aeration; root tips at the bottoms of the test-tubes must have had less thorough aeration than even those at the bottoms of the flasks that contained the largest volume of solution, and the tubes gave growth just as satisfactory as that shown by the flasks that contained the smallest volume. The low (and nearly equal) growth indices for test-tubes and for smallest-volume flasks are apparently not related to aeration conditions at all. They may have been due to inadequacy in the volume of solution in these instances; as though the 10 ml. of the solution used did not contain a sufficient supply of some substance or substances necessary for the maintenance of growth for two weeks under the prevailing conditions. It is also logically possible, of course, that the retarding effect of inadequate volume of solution may have been due to accumulation of injurious material resulting from the metabolism of the root tip, material that might become more widely dispersed in the larger solution volumes. Doubtless the aeration and volume relations of such cultures as these are themselves influenced by the growth rates of the excised root tips, by the length of the experimental period, by the prevailing temperature, and by other current environmental conditions.

Optimal conditional complexes for growth of the excised wheat root tips of this study, in the 2-week period uniformly employed, embraced redistilled water, the salts and salt proportions of the Uspenski solution, 20 gm.

of dextrose per liter of solution, yeast extract from 0.1 gm. of yeast for each liter of solution, a final or equilibrium H-ion concentration giving a pH value about 5.0, a maintained temperature of about 26°–27°, continuous electric light, and at least about 25 ml. of solution per culture of a single root tip. The numerical values from some cultures that gave remarkably extensive root systems under these conditions are given as follows:

Culture no.	I	II	III	IV	V	VI
Length of main root (mm.)	115	50	48	50	52	112
Total length of branch roots (mm.)	151	204	198	183	170	90
Growth index (mm.)	266	254	246	233	222	202

Culture no. 1 gave the greatest growth secured for any single root tip in any of the many cultures carried out under these conditions and for the 2-week experimental period. As is seen, the main root was 115 mm. long at the end of the period; there were five secondary roots totaling 133 mm. and a single tertiary root 18 mm. long. This represents a mean increment of root elongation of 19 mm. per day.

These experiments were carried out at the Boyce Thompson Institute for Plant Research in 1929–1930, while the writer held a National Research Council fellowship in botany. Laboratory facilities were kindly made available for this study by Dr. William Crocker, director of the Institute. The results were not assembled until the following year at the Pflanzenphysiologisches Institute of the University of Berlin, when the writer was a joint fellow of the National Research Council and the Rockefeller Foundation. In the course of the experimentation many helpful suggestions were received from Dr. L. O. Kunkel, of the Boyce Thompson Institute, and much critical assistance in the final writing of this paper was received from Professor Burton E. Livingston, of the Laboratory of Plant Physiology of the Johns Hopkins University.

Rockefeller Institute for Medical Research,
Princeton, New Jersey.

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RÔLE OF "NUTRILITES" IN THE NUTRITION OF MOLDS AND OTHER FUNGI

ROGER J. WILLIAMS AND J. MARSHALL HONN

Introduction

Since the discovery by WILDIERS (3) of the substance (or substances) of vitamin-like nature, to which he gave the name "bios," much work has been done on the subject of yeast nutrition. Especially were investigations along this line stimulated by the suggestion of the senior writer (5) that bios might be identical with one of the vitamins. It has been amply demonstrated, however, that bios is not a single substance (9) and that different strains of *Saccharomyces cerevisiae* require traces of different unknown substances for their growth stimulation (7). Likewise, whereas the term vitamin has been restricted in its meaning to include only substances involved in animal nutrition, and there is abundant evidence to indicate that various bacteria, fungi, etc., have requirements of a vitamin-like nature, the broad term "nutrilite" (6) has been suggested to cover the vitamin-like substances which are involved in the nutrition of organisms in general.

It was the purpose of the work here reported to investigate whether the fungi other than yeast, which can be propagated in the laboratory, have requirements for, or are stimulated by, nutrilites or vitamin-like substances.

In an early experiment with yeast, one of us observed accidentally that a certain mold seemed to produce a yeast-growth stimulant. Ordinary yeasts themselves apparently produce yeast growth stimulants slowly, and hence are not so greatly affected by the presence of nutrilites when relatively large quantities of yeast are planted. It was to be expected, therefore, that in the case of molds the effect of nutrilites would be apparent only when the original seeding of the mold in a synthetic medium was light. The case of molds is somewhat complicated also by the fact that they grow from spores, and these spores would be expected to contain a certain amount of stored nutrilites which would affect the early growth of the fungus.

WILLAMAN (4) found that *Sclerotinia cinerea,* the brown rot fungus of fruits, did not develop to maturity properly unless a fruit juice was added to the synthetic medium in which it was planted, and concluded that the addition was due to a vitamin-like substance. LEPESCHKIN (1) reported that vitamin B had some effect on the growth of *Penicillium glaucum* during the early stages. Aside from these cases, no work has been found dealing specifically with the requirements of molds for what we choose to call nutrilites.

In selecting the molds to be used, an attempt was made not only to include those of the more common varieties, but also to choose species that were representative of the different types, Eumycetes (*Penicillia* and *Aspergilli*) and Phycomycetes. In the preliminary work, various wild molds were obtained by exposing some of the control media to the laboratory air, and from other sources. All but two of these were discarded.

The ten different species of molds and three pathogenic yeasts used during the experiments are listed here and the source of each given. The numbers preceding the names designate the cultures from the sources indicated. Two additional molds, *Sclerotinia cinerea* and *Rhizopus nigricans*, from the American type culture collection were not cultured successfully.

1.	Blue-green mold (wild)	
2.	Light brown mold (wild)	
3.	*Penicillium roqueforti* (Roquefort cheese)	
1015.	*Aspergillus niger*	American type culture collection
1019.	*Aspergillus glaucus*	
1117.	*Penicillium expansum*	
1216.	*Mucor racemosis*	
Pathogenic molds:		
538.	*Penicillium crevicaule*	Dr. Fred Weidman, University of Pennsylvania
989.	*Microsporon fulvum*	
2058.	*Cephalasporium*	
Pathogenic yeasts:		
1871.	*Monilia penoyi*	Dr. Fred Weidman, University of Pennsylvania
1872.	*Monilia metalondinensis*	
1883.	*Monilia macedoniensis*	

All of these molds and yeasts were grown on the standard molasses agar medium (**10**) which has been used in this laboratory for a number of years for the cultivation of stock yeast cultures.

In order to carry out the experiments and answer the question just outlined, it was desirable to have a synthetic medium which contained all the substances which molds are known to require except those of a vitamin-like character. The medium used throughout these experiments (designated as control medium) had the following composition and was a modification of Czapek's medium, in that the ammonium-ion was added (**2**) in the form of phosphate, and small amounts of manganese and zinc were added because they have been found to be necessary for some fungi.

	Grams
Sodium nitrate, $NaNO_3$	3.0
Potassium phosphate, K_2HPO_4	0.50

Potassium chloride, KCl	0.50
Magnesium sulphate, $MgSO_4$	1.00
Ferrous sulphate, $FeSO_4$	0.015
Ammonium phosphate, $(NH_4)_2HPO_4$	0.50
Zinc sulphate, $ZnSO_4$	0.050
Manganese chloride, MnCl	0.010
Sucrose	30.0
Water	1000.0

For a source of nutrilites a solution containing 1.0 gm. per 500 cc. of an aqueous-alcohol extract of yeast (Northwestern) was used. One cc. of this solution was added to 10 cc. of the control medium in the following tests.

In order to be reasonably sure that the effect of the addition of this yeast extract was not due to the presence of ordinary nutrients in it (amino acids particularly), another "synthetic" solution containing a similar amount of known amino acids was prepared and used as an additional control. At the same time it was possible to ascertain the effect of this amino acid mixture alone on the growth of the fungi in question.

Equal amounts of the following pure amino acids were mixed and 1 cc. of a solution containing 0.1 gm. of mixed amino acids per 500 cc. was added to the control medium as indicated. (The amino nitrogen content of the yeast extract as determined by the method of Van Slyke was the same as that calculated for the amino acid solution.)

d-arginine monohydrochloride
l-proline
l-leucine
l-histidine dihydrochloride
d-lysine dihydrochloride
l-tryptophane

Experimentation

Erlenmeyer flasks of 50-cc. capacity, sterilized, and plugged with cotton were used as containers for the media. At each planting of the individual mold species, four flasks were employed into each of which were placed 10 cc. of the control medium. Into one flask containing 10 cc. of the control medium was placed 1 cc. of the amino acid solution, and the flask marked *A*. To a second flask containing 10 cc. of the control medium was added 1 cc. of yeast extract solution, and this was marked *Y*. Into a third flask was placed 1 cc. of each of the amino acid solutions and the yeast extract in addition to the control medium and this flask marked *YA*. The fourth flask contained only the control medium and was marked *C*. Thus for each planting four different media were used. A mold was planted in all of these four at the same time, and the rate and amount of growth and degree of maturity were noted from time to time.

To secure equal seedings in all media, the following method was developed for planting the molds. With a sterilized wire loop some spores were scraped from the surface of a stock culture and were shaken with a little distilled water until an even suspension was made. Enough spores were used to make the suspension cloudy, but in no case was the number great enough to cause visible cloudiness in the medium being seeded when 1 cc. of the suspension was planted in each flask. The plantings at various times were not exactly equivalent, of course, but for any one planting, the numbers of spores placed in the different flasks were made as nearly equal as possible. Some specimens produced few or no spores. In this case it was necessary, in order to make an even seeding, to shake bits of mycelium vigorously in water until the pieces were very small and equally distributed throughout the liquid.

The seeded flasks were placed in an incubator and kept at a temperature of 27°–29° C. Under these conditions growth was found to be considerably faster than when the cultures were exposed to the variations of room temperature. Higher temperatures than these caused evaporation of water, which condensing on the side of the flasks and running down, flooded the surface of the mold.

Observations were made about every two days, since in most cases the growth was not rapid enough for distinct changes to be seen within 24 hours. For the first day or two appearances were generally the same in all flasks, as the spores were germinating.

Some difficulty was encountered in making satisfactory estimations of the development of the mold at any time. Although the volume of the mold often appeared great, the actual dry weight of that from one flask would never be more than a few milligrams, and in many cases would be too small to weigh. It was necessary therefore to make some sort of comparative observation on the volume of growth. In the system used for this, a number was assigned to the amount of growth in one flask and if there was a larger growth in another flask, a higher number was assigned to it. If in one medium the growth was twice that in another, the number given to the first would be twice that given to the second, and so on. With a little practice the comparative amount of growth made in the different media could be estimated satisfactorily.

Some of the molds grew to greater volume before producing spores than did others. If the production of spores may be considered as an indication of the stage of maturity, then the volume of growth and the degree of maturity did not run parallel in all cases. For this reason it was thought desirable to maintain a separate record of the production of spores. The same system was used as in the record of growth.

The molds as a whole were found to behave rather erratically in their growth. A certain species might grow satisfactorily for several different plantings, and then after one particular planting it would suddenly die in the media when only a few days old. A new planting, however, would grow as before. It will be noted that during the third planting of no. 5, *Aspergillus glaucus,* the mold died in the *YA* medium. This did not happen to this mold in any of the other plantings. Such behavior might be due to bacterial contamination.

Tables I–IX contain the results of observations. The composition of the medium is indicated by the letters appended. *C* stands for control medium; *A* for medium to which amino acids have been added; *Y* for the medium to which yeast extract has been added; and *YA* indicates the medium which contained both yeast extract and amino acids in addition to the control medium. Owing probably to slight variations in the amounts planted and to the masking effect of stored food materials present in the spores, a single experiment often does not show striking results. However, the totals of the horizontal columns show the general behavior of the various molds in the different media.

The determinations of the amounts of growth of the yeasts (pathogenic) were made with a thermo-couple devised for use in measuring the turbidity of suspensions (**8**). The more turbid a suspension of the yeast, the smaller the galvanometer deflections. The galvanometer readings have been translated into milligrams of moist yeast per cubic centimeter.

The results of the tests are summarized as follows:

1. Growing as well in the control medium as when additions were made: blue-green mold (wild), *Penicillium roqueforti, P. crevicaule.*

2. Stimulated in growth by amino acids: light brown mold (wild), *Aspergillus niger, A. glaucus, Penicillium expansum, Mucor racemosis, Microsporon fulvum, Cephalasporium.*

3. Stimulated in growth by yeast extract: light brown mold (wild), *Aspergillus niger, Mucor racemosis, Microsporon fulvum, Cephalasporium, Monilia metalondinensis, M. macedoniensis.*

4. Yeast extract producing distinctly more growth stimulation than did the amino acids: light brown mold (wild), *Aspergillus niger* (?), *Mucor racemosis, Microsporon fulvum, Cephalasporium, Monilia metalondinensis, M. macedoniensis.*

5. Spore formation stimulated by amino acids: blue green mold (wild), light brown mold (wild), *Aspergillus niger, A. glaucus, Penicillium expansum.*

TABLE I

GROWTH AND SPORE FORMATION, BLUE-GREEN MOLD (WILD)

	DAYS																TOTAL
	3	5	7	2	7	3	5	2	4	6	3	5	7	1	2	3	
GROWTH																	
C	..	2	5	1	5	2	5	1	3	3	4	6	7	2	2	2	50
A .	1	4	4	1	5	2	3	1	3	5	4	5	6	2	2	2	50
Y . ..	..	..		2	5	1	5	1	5	6	2	4	4	3	4	4	46
YA	1	3		2	×*	2	4	1	4	5	2	3	4	3	4	4	42
SPORE FORMATION																	
C . .	.	.	5	..	3	.	5	.		1	1	2	7	.	.	..	24
A	.	3	4		3	..	3		1	5	2	3	6	.		..	30
Y	..	..		...	5	..	5		5	6	1	1	2				25
YA	..	2	5			..	3		2	5	1	4	4			1	27

* × indicates death of mold.

TABLE II

GROWTH AND SPORE FORMATION, LIGHT BROWN MOLD (WILD)

	DAYS																			TOTAL
	3	5	13	14	3	5	7	4	6	2	3	5	1	3	4	5	3	5	7	
GROWTH																				
C . .		1	1	1	1	1	2	1	1	1	2	3	1	2	2	2	3	4	4	33
A . .	..	1	2	2	1	3	3	1	2	2	2	3	1	4	4	4	5	6	8	54
Y ...	1	2	2	3	2	4	5	2	4	3	3	4	1	4	5	5	2	4	7	63
YA	1	3	6	8	2	4	6	2	2	4	4	5	1	4	4	5	4	7	6	78
SPORE FORMATION																				
C	..	..	.			..	...	.	...			..	..	...	..	...	...	1	1	2
A	.	.	.	...	...	..	...	...	...	.	.	...	..	..	...	...	...	2	6	8
Y	...	.	.	.	...	..	..	...	..	.	.	2	.	...	...	..	..	1	1	4
YA	..	..	3	4	..	.	...	...	..		2	4	..	.		..	.	4	7	24

TABLE III

GROWTH AND SPORE FORMATION, PENICILLIUM ROQUEFORTI

	Days							Total
	1	5	2	7	3	5	7	
				Growth				
C	1	5	3	6	4	4	4	27
A	1	4	2	6	3	5	5	26
Y	1	2	1	6	2	4	4	20
YA	1	1	1	6	2	4	4	19
				Spore formation				
C	.	4		4	..	2	3	13
A	.	2		4	..	4	5	15
Y				6	..	2	3	11
YA				5	.	2	4	11

TABLE IV

GROWTH AND SPORE FORMATION, ASPERGILLUS NIGER 1015

	Days								Total
	3	5	2	2	6	1	2	4	
				Growth					
C	5	5	4	2	2	1	1	2	22
A	5	6	4	2	2	2	2	4	27
Y	4	4	4	2	4	3	4	5	30
YA	5	6	4	2	4	4	5	6	36
				Spore formation					
C	3	3	3	2	2	.		1	14
A	5	6	3	2	2		.	2	20
Y	2	2	2	2	4	.		4	16
YA	4	5	3	2	4	...	.	6	24

TABLE V

GROWTH AND SPORE FORMATION, ASPERGILLUS GLAUCUS 1019

	DAYS															TOTAL
	3	5	2	3	1	3	4	5	8	3	5	7	2	3	5	
	GROWTH															
C	3	4	2	6	1	4	7	8	8	3	6	10	2	2	2	68
A	3	5	2	6	2	5	8	8	8	6	6	10	2	3	3	77
Y	2	3	3	5	3	2	4	4	8	4	6	10	3	4	5	66
YA	2	3	3	5	4	4	×*	×	×	4	4	6	4	4	6	49
	SPORE FORMATION															
C	1	1		3	.	2	2	2	2	2	2	3		1	1	22
A	1	1		3	..	4	5	5	5	2	2	3		2	2	35
Y	2	2		2			5	5	5	.	.	2	1	2	2	28
YA	2	2	.	2		..	.		.	2	3	4	2	4	4	25

× indicates death of mold.

TABLE VI

GROWTH AND SPORE FORMATION, PENICILLIUM EXPANSUM 1117

	DAYS																	TOTAL
	3	5	7	2	4	1	3	4	5	3	5	7	3	6	2	3	5	
	GROWTH																	
C	2	3	4	3	8	1	3	4	4	3	2	×	2	2	2	2	×	46
A	3	3	5	3	8	2	4	4	4	2	2	4	3	4	3	3	×	58
Y	1	3	3	1	7	3	3	3	3	1	1	3	4	5	3	4	×	48
YA	1	3	3	1	4	4	3	3	3	1	2	6	6	8	3	4	×	55
	SPORE FORMATION																	
C	...	2	3	.	8	.	3	4	4	.	...	..	...	.	...			24
A	...	3	5	..	7	..	4	5	5	.	..	..	..	..	.	...	..	29
Y	..	1	1	..	4	.	1	3	3		..	..	...	1	..	..	.	14
YA	...	1	1		1	..	..	..	..	..	.	...	4	6	..	..	.	13

TABLE VII

GROWTH AND SPORE FORMATION, MUCOR RACEMOSIS 1216

	DAYS						TOTAL
	1	2	3	3	6	3	
			GROWTH				
C	1	1	2	2	2	2	10
A	2	2	4	2	2	3	15
Y	3	3	4	4	5	4	23
YA .	4	4	4	3	4	3	22
			SPORE FORMATION				
C	..	.	.	1	2	1	4
A	.		..	...	..	1	1
Y			...	2	4	3	9
YA	..	.	..	...	3	2	5

TABLE VIII

GROWTH OF THREE PATHOGENIC MOLDS

				DAYS										
NO. 538, PENICILLIUM CREVICAULE			NO. 989, MICROSPORON FULVUM								NO. 2058, CEPHALASPORIUM			
	3	7		1	2	5	7	1	3	TOTAL	1	4	6	TOTAL
M	1	2	C	2	4	5	6	0	2	19	2	2	6	10
A	1	×*	A	2	4	5	6	2	4	23	3	4	6	13
Y	1	×	Y	3	5	6	6	2	5	27	4	6	6	16
YA	1	2	YA	4	5	7	7	2	6	31	4	6	6	16

* Indicates death of mold.

6. Spore formation stimulated by yeast extract: *Mucor racemosis.*

7. Growth inhibited by amino acids: none.

8. Growth inhibited by yeast extract: blue-green mold (wild), *Penicillium roqueforti.*

9. Spore formation inhibited by amino acids: *Mucor racemosis* (?).

10. Spore formation inhibited by yeast extract: *Penicillium expansum.*

11. Both amino acids and yeast extract necessary for spore formation: light brown wild mold (striking).

TABLE IX

GROWTH OF THREE PATHOGENIC YEASTS

No. 1872, MONILIA METALONDIENSIS

	HOURS			TOTAL
	48	30	36	
C	0.78	0.67	0.52	1.97 mg. moist yeast per cc.
A	0.87	0.65	0.48	2.00
Y	1.35	0.72	0.90	2.97
YA	1.55	0.77	0.78	3.10

No. 1883, MONILIA MACEDONIENIS

C	0.26	0.30	0.26	0.82 mg. moist yeast per cc.
A	0.31	0.30	0.26	0.87
Y	0.60	0.72	0.50	1.82
YA	0.74	0.77	0.63	2.14

No. 1871, MONILIA PENOYI

	DAYS			
	2	4	2	
C	2	×*	2	
A	2	×	2	
Y	2	×	2	
YA	2	×	2	

* Indicates death of yeast.

Discussion

It has been found that the molds are rather difficult to work with and rather erratic in their behavior. Mixing of one mold with another, presumably, because of the activity of "mites" which crawl through the cotton plugs carrying spores with them, is one difficulty that had to be met, in addition to the difficulty of keeping spores in general from becoming dusted into all utensils. Bacterial contamination may enter into some cases, although the usual bacteriological technique was used throughout in handling the organisms. Wherever a foreign organism appeared to be present, the culture was discarded.

In spite of the irregularities of behavior, however, there is evidence that a number of molds and two or three yeasts tested (*Monilias*) are stimulated in their growth by vitamin-like substances present in yeast extract. It will be noted that the physiological actions of the yeast extract and the amino acid solution are both definitely positive but that they are distinct. As might be expected, the formation of spores with their relatively high protein content is stimulated in a number of cases by the presence of amino acids which are apparently synthesized with difficulty by several molds. Yeast extract has comparatively little effect in this regard. In stimulating growth, however, yeast extract has a greater effect than the amino acid solution in the case of seven organisms. The striking case of the light brown wild mold is worthy of note, inasmuch as both yeast extract and amino acids together are necessary for appreciable spore formation.

There appear to be more or less specific poisonous substances in the yeast extract which inhibit the growth of certain molds. Such substances are not in the amino acid mixture. We have previously observed in studies on yeast the existence in certain extracts of poisons which are effective toward certain strains of yeast and not toward others (**9**).

It was interesting to note that some of the molds seemed to be able to adapt themselves to the medium on which they were growing. This was particularly noticeable in the case of the light brown (wild) mold. After successive cultivations in the control medium this mold grew in this medium better than previously. This made it necessary to grow all stock cultures on a standard medium, as indicated previously.

The situation with respect to molds and their relationship to various nutrients seems to be fairly clear. Apparently for carrying on the living activities of an individual fungus, certain chemical materials are indispensable. Some of these, such as the inorganic ions and carbohydrate (or substitute) material, must be supplied in the food and cannot be produced by the organism. Other of the necessary cell constituents can be built up with varying ease. In the case of some molds (*Penicillium roqueforti* for example), apparently the protein building stones are built up with great ease, since introduction of amino acids into the medium has no apparent effect on the growth of the mold. On the other hand, such molds as *Penicillium expansum* were definitely stimulated in their growth by amino acids, and in spore formation a number were helped by the presence of the protein building stones.

The fact that in certain cases amino acids stimulate growth or spore formation does *not* mean, however, that the fungi in question are wholly unable to synthesize the amino acids. It merely means that synthesis does not take place with greatest ease.

Likewise with the nutrilites or vitamin-like substances which are apparently also necessary cell constituents: the fact that they stimulate growth does not mean that they cannot be synthesized by the organism, but rather that they cannot be synthesized with ease. Accordingly, when presented to the organism ready-made, growth is enhanced.

In spores undoubtedly the necessary cell constituents are all stored, and for a time the organism is able to grow on this stored material. Accordingly, the growth at first is apparently the same whether nutrilites and amino acids are furnished in the medium or not. It is during the second phase of growth that the effect of nutrilites becomes apparent in a number of cases. If left in the same medium for a long time, the mold eventually (though slowly) produces enough of the essential organic cell constituents so that the whole of the food material may be used and the final growth may be approximately the same, whether or not nutrilites were present in the medium. In some cases, where the nutrilites are synthesized by the organism with great difficulty, the growth may continue to be very slow. There is the possibility, already mentioned, however, that the organism may adapt itself to a medium and increase its synthetic powers.

That certain molds are capable of producing substances belonging to the category of nutrilites was confirmed in this laboratory in connection with this study, inasmuch as a culture of *Aspergillus niger* grown on a synthetic medium was extracted and found to contain the nutrilite or "bios" for WILDIERS' yeast.[1]

Summary

Ten molds and three yeasts (*Monilias*) were cultured in various media, and from the study the following principal conclusions are drawn:

1. Several molds are apparently stimulated in their growth by substances of vitamin-like character which we have called nutrilites.

2. A number of molds sporulated much more freely when growing in a medium containing a mixture of pure amino acids.

3. The effects of nutrilites (and amino acids) on mold growth are masked in part by the presence of stored materials in spores. Yeasts respond more readily to the presence of nutrilites in the medium than do molds.

4. The fact that growth is stimulated by the presence of certain indispensable substances does not mean that the organism is unable to synthesize them, but rather that synthesis takes place with difficulty and consequent slowness. Molds, in common with yeasts, are in general apparently able to synthesize nutrilites slowly.

UNIVERSITY OF OREGON,
EUGENE, OREGON.

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FURTHER NOTES ON THE NUTRIENT REQUIREMENTS AND THE HISTOLOGY OF THE CRANBERRY, WITH SPECIAL REFERENCE TO THE SOURCES OF NITROGEN[1]

RUTH M. ADDOMS AND F. C. MOUNCE

(WITH TWO FIGURES)

Introduction

A project concerned with the nutrient requirements and the histology of the cranberry (*Vaccinium macrocarpon* Ait.) has been in progress at the New Jersey Agricultural Experiment Station for two years. Preliminary experiments (**1**) showed that the cranberry plant is capable of utilizing ammonium nitrogen. After these experiments were initiated, TIEDJENS and ROBBINS (**12**) showed that for tomato and certain other crop plants either ammonium or nitrate was a favorable source of nitrogen, but that the ability of the plants to utilize these different sources of nitrogen varied with the hydrogen-ion concentration of the nutrient medium, nitrate being superior to ammonium in a decidedly acid nutrient solution (pH 4 or 5) whereas ammonium was superior to nitrate in a neutral or slightly alkaline solution (pH 7 and above). The present investigation was therefore undertaken with the special object of determining the ability of the cranberry plant to utilize different sources of nitrogen at various hydrogen-ion concentrations. The problem is of practical importance since cranberries are grown commercially in bog soils of high acidity.

Methods

CULTURAL

The plants selected for these experiments were of the Howe variety, chosen as preferable to the Early Black of previous experiments (**1**) because of their larger size and more vigorous growth.[2] In the fall of 1930 several hundred one-year-old rooted cuttings were selected for uniformity; the roots were washed carefully, the plants set in 8-inch pots of washed quartz sand, and the pots buried in sand in a cold frame and covered with straw and brush. Throughout the winter, water was applied at regular intervals. At the onset of warm weather in the spring, the potted plants were removed to a refrigeration room, where they were maintained at a temperature slightly below 0° C. for a month, until such time as it was practicable to start experiments in the greenhouse.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station.

[2] The plants were obtained through the courtesy of Miss ELIZABETH C. WHITE, of Whitesbog, N. J.

On June 22, 1931, most of the plants were transferred to 3-gallon glazed porcelain crocks filled with washed quartz sand, five or six plants to a crock. They were then divided into three series, which until the latter part of August were kept in the greenhouse and received the following nutrient treatments.

SERIES 1.—Fifteen crocks received a complete nutrient solution containing nitrogen in the form of calcium nitrate. The solution R_2S_2 of previous experiments (**1**) was selected because it has been shown to support vigorous vegetative growth.

SERIES 2.—Fifteen crocks received a complete nutrient solution containing nitrogen in the form of ammonium sulphate. This solution also had been shown to support vigorous vegetative growth.

SERIES 3.—Fifteen crocks received nitrogen in the form of glycine. Calcium nitrate of the solution of series 1 was replaced by calcium chloride and an equivalent amount of nitrogen was supplied as glycine.

The composition of the various solutions is shown in table I. All had a total osmotic concentration of approximately one atmosphere. Iron was supplied from time to time as ferrous sulphate. In each series the fifteen crocks were divided into three groups, which received the nutrient solution at an initial hydrogen-ion concentration of 4, 6, and 8 respectively, the

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS USED

SERIES	SALT	PARTIAL VOLUME-MOLECULAR CONCENTRATION
Nitrate	KH_2PO_4	0.0049
	$Ca(NO_3)_2$	0.0049
	$MgSO_4$	0.0099
Ammonium	KH_2PO_4	0.0020
	$CaCl_2$	0.0073
	$(NH_4)_2SO_4$	0.0014
	$MgSO_4$	0.0071
Glycine	KH_2PO_4	0.0049
	$CaCl_2$	0.0049
	$MgSO_4$	0.0099
	$CH_2 \cdot NH_2 \cdot COOH$	0.0032

reaction being adjusted by the addition of potassium hydroxide or sulphuric acid. Each crock received daily 1 liter of nutrient solution, which for the pH 4 and 6 cultures was supplied by SHIVE's constant-drip method (**11**), and for the pH 8 cultures (because of precipitation) was mixed in a jar and applied twice daily. All of the cultures were flushed with distilled water once a week.

In addition to the three series of sand cultures, two series of soil cultures were set up. Plants similar to those of the sand cultures were transplanted to bog soil in 8-inch clay pots. Nine pots received nitrogen in the form of calcium nitrate and nine in the form of ammonium sulphate. To three pots of the nitrate series and to three of the ammonium series were added 80 grams of lime, to three were added 40 grams and to three no lime was added.

HISTOLOGICAL AND MICROCHEMICAL

Plants from the cultures just described furnished material not only for study of general growth responses, but also for microscopic examination. Most of the microchemical tests employed were suggested by ECKERSON (**2**) of the Boyce Thompson Institute. For both histological and microchemical work fresh material was employed; although the endophytic fungus associated with the growth of the cranberry plant (**10**) is extremely small, it can be observed in fresh material, especially if stained lightly with ruthenium red, cotton blue, or mercurochrome.

Results

CULTURAL

In none of this season's sand cultures did linear growth of stems equal that of the previous season (**1**), for the cuttings were transferred to sand cultures and supplied with nutrient solutions much later; and prior to being transferred they were in a very inactive state, with the result that they were slow in resuming rapid growth. However, all of the plants of this season's series were on a par when they were set out, and within a few days all had started to produce new stem growth. Thus the comparative rates of growth in the different cultures are of significance. All of the plants produced some increase in stem length, although in the $\frac{\text{glycine}}{\text{pH 8}}$ and the $\frac{\text{nitrate}}{\text{pH 8}}$ cultures the increase was very slight, being limited to the initial growth just mentioned. By the middle of July decided differences were noticeable. The $\frac{\text{glycine}}{\text{pH 8}}$ and the $\frac{\text{nitrate}}{\text{pH 8}}$ plants showed a chlorosis from which they failed to recover upon the addition of either iron or boron. By

July 30 the $\frac{\text{glycine}}{\text{pH 8}}$ plants were so nearly dead that they were discarded, and the $\frac{\text{nitrate}}{\text{pH 8}}$ plants followed on August 6.

Of the nitrate cultures that persisted after August 6, all showed less growth than either the ammonium or the glycine cultures. There was little difference between those at pH 4 and those at pH 6; all were dark green and apparently healthy, those at pH 4 slightly more so than those at pH 6, but none was vigorously vegetative. When linear growth of stems was measured on August 24, there was very little difference between them. These results, as well as those in the other sand cultures, are shown in table II.

TABLE II

Linear growth of stems in sand cultures, June 27 to August 24

Culture	Linear growth of stems (average of 25 plants) in cm. per plant
	cm.
Ammonium, pH 4	36
Ammonium, pH 6	88
Ammonium, pH 8	81
Nitrate, pH 4	34
Nitrate, pH 6	30
Glycine, pH 4	86
Glycine, pH 6	69

The glycine plants at pH 4 and pH 6 grew well, as did the ammonium plants at pH 6 and pH 8. All of these plants were dark green, produced new stem growth, had apparently healthy root systems, and presented an appearance that in all respects was satisfactory to a commercial cranberry grower. Over a period of several weeks the $\frac{\text{glycine}}{\text{pH 4}}$ plants were superior in appearance to any of the other plants in sand culture, although by August 24 little or no difference was evident between them and the ammonium plants at either pH 6 or pH 8. The $\frac{\text{ammonium}}{\text{pH 4}}$ plants remained stunted, and ultimately showed an injury to the stem tip that the addition of manganese and boron did not alleviate; they were discarded on August 26.

On August 27, stem tips and leaves from the most vigorously growing sand culture of each series were analyzed for alpha-amino nitrogen and for ammonia. The results, computed on a green weight basis, are as follows:

PLANTS	α-AMINO N	AMMONIA
$\frac{\text{ammonium}}{\text{pH } 8}$	0.031%	0.0053%
$\frac{\text{glycine}}{\text{pH } 4}$	0.040	None
$\frac{\text{nitrate}}{\text{pH } 4}$	0.017	None

Figure 1 shows the various cultures as they appeared on August 5. As stated, many of the cultures produced a considerable amount of new stem growth after that date.

Of the soil series, several cultures produced excellent stem growth and dark green leaves. Of these cultures the ammonium plants that had been supplied with a medium quantity of lime stood first, both in general appearance and in amount of linear growth of stems. Several of the plants retained after the conclusion of the experiments were growing vigorously at the end of six months. Second in amount of linear growth were the ammonium plants that had received no lime and the nitrate plants that had received a medium amount of lime. The others, ammonium plants receiving a large quantity of lime and nitrate plants with medium and high quantities of lime, were far less vigorous in growth, although they were not markedly chlorotic and during the course of the experiment it was not deemed necessary to discard them.

To furnish supplementary evidence, plants several years old were collected from bogs and brought to the laboratory for chemical analysis. All were of the variety Early Black, but they differed decidedly in vegetative vigor, according to the nutrient conditions of the bogs in which they had been grown. Some plants were highly vegetative (although fruitful), with long succulent shoots and green leaves. Others were much less vigorous, with shorter and more woody shoots, and many of the leaves were red with anthocyanin pigments; microscopic examination showed a much larger proportion of thick-walled cells, a heavier cuticle, and larger deposits of oil. A third group seemed intermediate between the other two in regard to the degree of vegetative vigor, although more nearly like the weakly vegetative than the strongly vegetative group.

Leaf blades only were analyzed. Amounts of ether extract, on the basis of dry weight, were as follows:

Strongly vegetative plants	10.57	per cent.
Intermediate plants	12.42	" "
Weakly vegetative plants	14.58	" "

HISTOLOGICAL AND MICROCHEMICAL

A previous paper (**1**) describes the stem and root structure of the cranberry plant, and includes some discussion of the distribution of the endophytic fungus in these organs.

In this season's work, the endophytic fungus was found in all plants of both sand and soil cultures, both in association with the roots and distributed through the entire stem system. A decided difference in amount of mycelium was noticed, however, between the rapidly growing plants and those that were stunted and chlorotic. In all of the rapidly growing plants, such as those grown with $\frac{\text{glycine}}{\text{pH } 4}$, the cells of the stem tip, even embryonic cells, were crowded with mycelium. Slightly farther back the mycelium was especially evident in pith, although it could be observed in all types of cells, including the epidermis. Many of the hyphae had swollen tips (fig. 2), which did not stain with either ruthenium red or cotton blue, but which showed a high content of protein and a few oil globules. Tests with gold chloride and with iodine-ammonia indicated an appreciable content of tannin in these swollen tips as well as in the cells of the cranberry stem. JANSE (**4**) figures apparently similar structures in the mycorrhiza of certain Javanese plants. In the more mature portions of the stem, mycelium was observed in various stages of disintegration, as previously described (**10, 1**). Within these masses of disintegrating material there could often be observed conspicuous granules varying in color from brown or black to a deep cherry red. They were sometimes evident in cells in which strands of mycelium were still apparently intact.

On the other hand, the non-vigorous stunted plants, such as the $\frac{\text{nitrate}}{\text{pH } 6}$ ones, contained comparatively little mycelium at any time during the season's growth. Mycelium could be distinguished in the stem, but not abundantly, and most of it was in the granular stages of disintegration, even near the growing tip. Much the same condition had been noted in the dormant cuttings at the initiation of the experiments on June 22.

As in the plants of previous experiments (**1**), nitrates could not be detected at any time in either the fungus or the cranberry plants of any cultures. By means of the Nessler test and the formation of ammonium chloroplatinate crystals, ammonium could be detected in the tissues of the plants to which it had been supplied in the nutrient solution. It was especially evident within a few hours after a plant that had grown in a defi-

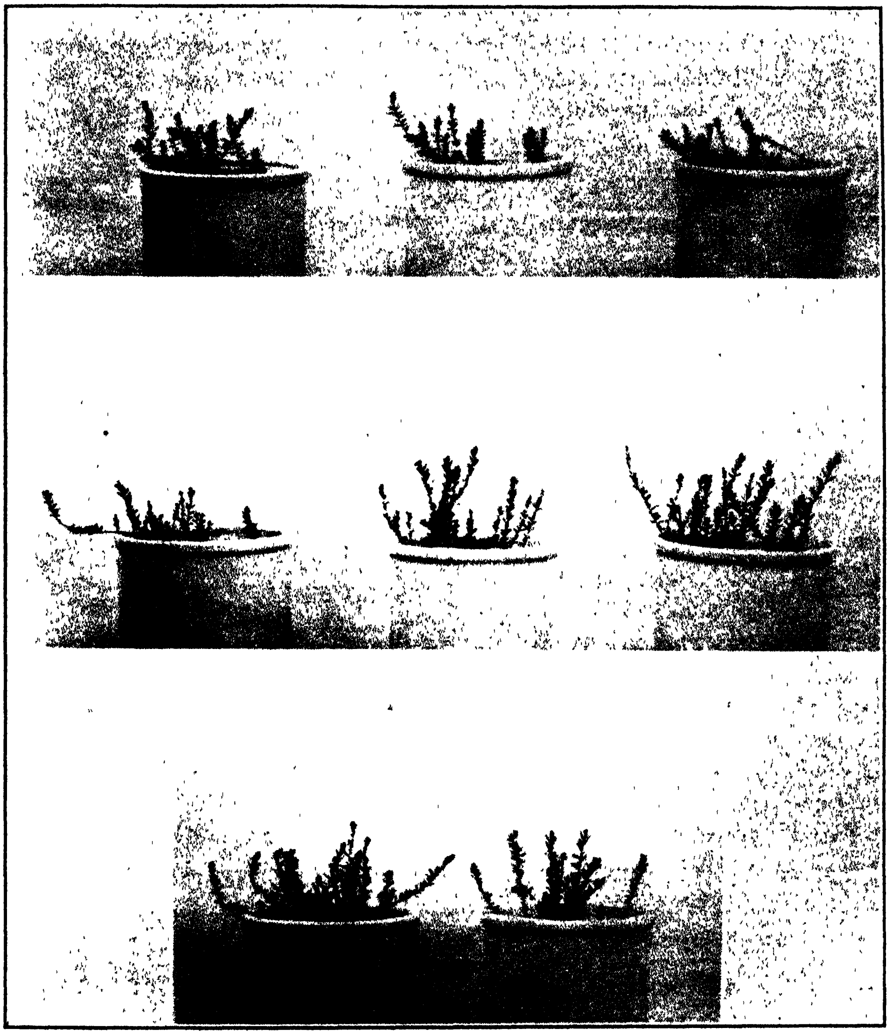

FIG. 1. Plants of sand cultures as they appeared on August 5, 1931: top (left to right), nitrate cultures at pH 4, 6, and 8 respectively; middle (left to right), ammonium cultures at pH 4, 6, and 8 respectively; bottom (left to right), glycine cultures at pH 4 and 6 respectively. × 1/11.

ciency of nitrogen was transferred to an ammonium-containing nutrient solution.

Glycine was apparently present in the stems of plants to which it had been supplied in the nutrient solution. To fresh sections of young stem, absolute alcohol was added. Within an hour there appeared very small crystals, which in the course of 12 hours increased greatly in size. With the exception of a few crystals that were obviously of some other substance, they had the crystalline form of glycine, and when immersed in oily liquids of the several refractive indices of glycine (**5**), the respective facets of the crystals disappeared. These facts would seem substantial evidence that the crystals were of glycine. They were present in large numbers in the young stems of the $\frac{\text{glycine}}{\text{pH 4}}$ plants, especially in the outer cells of the pith. A few were present in the mycelium in these sections. In the $\frac{\text{glycine}}{\text{pH 6}}$ plants a somewhat lower concentration of glycine was indicated; in the $\frac{\text{ammonium}}{\text{pH 8}}$ plants a few crystals were present; and in the nitrate plants there were practically none.

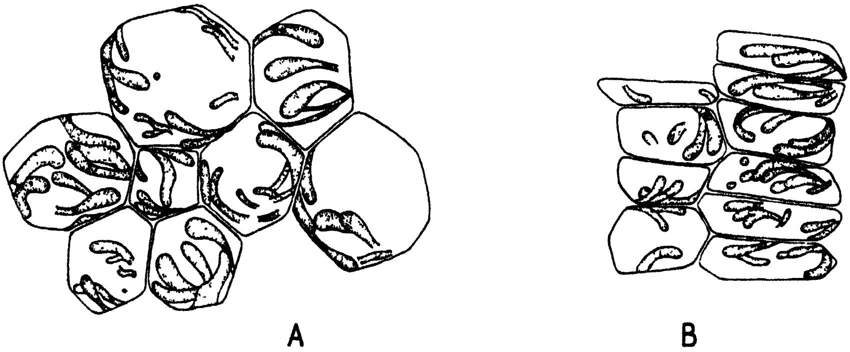

FIG. 2. Cells from stem tip of strongly vegetative $\frac{\text{glycine}}{\text{pH 4}}$ plant, showing swollen tips of hyphae: *A*, as seen in cross-section of stem; *B*, as seen in longitudinal section. × 600.

Discussion

From the present experiments, as well as earlier work (**1**), it is evident that in the cranberry, as in many other plants that have been studied by other workers (**6, 7, 8, 9**), degree of vegetative vigor is associated with the nitrogen metabolism of the plant. In the cranberry, however, food is stored not only as starch but also as oil; in the leaves, starch seems to be transitory and most of the stored food is in the form of oil, whereas in the stem large deposits of starch may be found. Chemical analyses of bog plants show that there is a definite correlation between degree of vegetative vigor and percentage of fats and lipoids in leaf blades (page 648), the percentage of ether-soluble substances being lowest in the most vigorously vegetative plants.

The cultures of these experiments, both sand and soil, indicate that young cranberry plants are capable of utilizing a variety of different substances as sources of nitrogen, but that the ability to use different substances varies with the reaction of the nutrient solution. In sand cultures nitrates can be used to at least fair advantage in acid but not in alkaline reaction, while ammonium can be utilized in neutral or alkaline but not in highly acid reaction. These results are in general agreement with those of TIEDJENS and ROBBINS (**12**) for tomato and other plants, although apparently the cranberry can utilize ammonium in somewhat more acid reaction than could the plants with which they worked. The findings are interesting in relation to the fact that cranberries are grown commercially in bog soil that is decidedly acid, even more so than the pH 4 cultures of these experiments, and that the most commonly used nitrogenous fertilizer is sodium nitrate. It might be expected, then, that the bog soil cultures to which ammonium sulphate was added would be inferior to the nitrate pots. Quite the opposite was true, however, for the ammonium cultures, both with and without lime, produced stem growth exceeding that of any of the nitrate soil cultures. The most plausible explanation of the difference between soil and sand cultures lies in the greater buffer properties of the bog soil. The present experiments did not include the application of ammonium sulphate over a period of several years.

As stated, the ammonium ion seemed to be readily absorbed by the cranberry plant (or its endophyte), and could be detected in its tissues; whereas nitrates could not be detected in either the cranberry or its endophyte at any time, indicating that if nitrates are absorbed as such, they must be converted into other substances immediately. Moreover, bog soil is usually low in nitrates.

It is generally assumed that one of the first steps in the synthesis of proteins is the reduction of nitrates to nitrites and ammonia (**3**). If this be true, it would seem reasonable to suppose that under proper environmental conditions the ammonium ion should furnish a more readily available source of nitrogen than does the nitrate ion. Such a conclusion is borne out by experiment. It has been observed repeatedly in this laboratory that the addition of ammonium salts to plants low in nitrogen produces more rapid response than the addition of nitrates. One commercial grower, reporting the application of ammonium sulphate to a cranberry bog over a period of several years, states that at first it produced greater vegetative growth than either sodium nitrate or dried blood, and that the leaves were dark green; but that after ammonium sulphate had been applied for several years the foliage became noticeably lighter, there was a decrease in the amount of fruit produced, and the berries were of poor color and keeping quality. It is apparent that the symptoms are those of an excess of nitrogenous fertilizer, resulting in excessively vigorous vegetative growth and ultimately in a decreased production of fruit. The cranberry seems to be more sensitive than the tomato and certain other plants to an oversupply of nitrogen. It should be remembered also that not only is ammonium probably a more quickly available form of nitrogen than is nitrate, but that the amount of nitrogen in a molecule of ammonium sulphate is approximately 25 per cent. more than in a molecule of sodium nitrate, necessitating the use of a smaller quantity of the salt as fertilizer.

Several of the plants that remained from sand-culture experiments reported in a previous paper (**1**), and that had been stored in a cold frame through the winter and spring, were removed to the greenhouse on July 22 and supplied with the nutrient solution containing ammonium sulphate as a source of nitrogen, at pH 6. New stem growth became evident within two days, and within a week there were many new shoots two inches or more in length. Throughout the remainder of the season (their second season with ammonium as the only source of nitrogen) growth was vigorous and apparently healthy. It would thus seem sure that for cranberry, as well as for tomato and other plants reported by TIEDJENS and ROBBINS (**12**), ammonium in itself is not toxic, but only demands a satisfactory reaction. Nitrates could not be detected in any of the ammonium drips at any time, thus apparently precluding the possibility of nitrification of the ammonium in the soil. Macrochemical analysis (page 647) showed a considerably higher concentration of alpha-amino nitrogen in the plants that had been supplied with ammonium than in those that had been supplied with nitrate. These results are in excellent agreement with those of TIEDJENS and ROBBINS (**12**) and suggest that the ammonium absorbed was rapidly converted into amino acids within the plants.

According to the generally accepted theories of protein synthesis, proteins are built up from amino acids. If, then, it were possible for a plant to absorb amino acids, they should be a readily available source of nitrogen. Preliminary experiments (**1**) suggested this to be true. The glycine series of the present experiments has yielded further evidence. Not only did the glycine cultures produce vegetative growth, but over a period of several weeks the $\frac{\text{glycine}}{\text{pH 4}}$ plants were superior in appearance to any of the other sand cultures, and when linear growth was measured on August 24 they were found to have produced practically as much stem growth as the best ammonium cultures, and more than any of the nitrate cultures.

It may be suggested that within the soil solution of these cultures glycine was transformed to ammonium, and that as ammonium it was absorbed by the plant. To be sure, the drip from these cultures was found to contain ammonium, but only in extremely small quantities. Moreover, the plants were growing better with glycine as a source of nitrogen than with ammonium at the same hydrogen-ion concentration. Also a far greater concentration of glycine was detectable as crystals within the plant when glycine was present in the external medium than when it was not. It thus seems reasonably sure that the cranberry plants of these cultures were dependent upon glycine as a source of nitrogen, not upon substances that were formed from glycine.

How much of the plant's ability to utilize glycine is attributable to the activities of its endophytic fungus has not been determined. By way of comparison, several tomato plants were grown in sand culture supplied with nutrient solution containing nitrogen in the form of glycine, at pH 4, 6, and 8. Over a period of several weeks all of these plants grew with vigor and produced good fruit. Glycine was detected microchemically in both roots and stems. It would thus seem that green plants, irrespective of mycorrhiza, may under certain conditions be able to utilize amino acids as a source of nitrogen. It is possible that the success of dried blood as a fertilizer for cranberries lies in the fact that upon disintegration it may yield amino acids and ammonium.

In all of the vigorously growing sand cultures it was noticed that mycelium of the endophytic fungus was very conspicuous in the tissues, especially the parenchymatous tissues of the young stems, penetrating even the embryonic tip (page 648). In all of the less vigorous cultures, whether of the nitrate, ammonium, or glycine series, far less mycelium was evident, and most of it was in the granular stages of disintegration. Essentially similar results were noted in the minus-nitrogen series of earlier experiments (**1**). Considered collectively, these observations suggest that the amount of mycelium of the endophytic fungus in the stem tissues varies more or less directly with the vigor of vegetative growth.

The present experiments tend to substantiate a conclusion that has been gathering form for several years as a result of various pieces of work, namely, that if under two sets of environmental conditions plants are produced which in all visible respects resemble each other, these plants will not differ materially in histological structure or in content of elaborated materials. There may be minor differences, such as the concentration within the plant of certain mineral salts or their ions that are supplied in the soil solution. For example, in the $\frac{\text{glycine}}{\text{pH } 4}$ and $\frac{\text{glycine}}{\text{pH } 6}$ plants of the current experiments, more glycine can be detected than in the plants of the other series; also, ammonium can be detected in plants of the ammonium series but not of the others. Except for such minor differences, it would be impossible to distinguish histologically or microchemically between the most vigorously growing cultures of the glycine and of the ammonium series. Both could easily be distinguished from the best cultures of the nitrate series by their more vigorous growth, as well as by histological differences; but the nitrate cultures would be found to differ little histologically or chemically from the less vigorous cultures of the ammonium series.

Summary

1. Young cranberry plants were grown for two months in sand cultures supplied with nutrient solutions containing nitrogen in the form of calcium nitrate, ammonium sulphate, and glycine respectively. Each of the three series received the nutrient solution at three different hydrogen-ion concentrations, *viz.* pH 4, 6, and 8.

2. The plants that received ammonium at pH 6 and pH 8, and those that received glycine at pH 4 and pH 6, grew most vigorously, producing long stem growth, dark green foliage, and apparently healthy root systems. The $\frac{\text{ammonium}}{\text{pH 4}}$ and the $\frac{\text{glycine}}{\text{pH 8}}$ plants were stunted and chlorotic.

3. The nitrate cultures producing the greatest stem growth were at pH 4. However, all of the nitrate cultures were inferior in vegetative growth to the glycine cultures at pH 4 and pH 6 and the ammonium cultures at pH 6 and pH 8.

4. It is evident from these experiments that the cranberry can utilize several different sources of nitrogen, including nitrate, ammonium, and glycine.

5. The results of the present experiments confirm the findings of TIEDJENS and ROBBINS with certain other plants, that in sand cultures nitrate can be utilized to best advantage in acid solutions, and ammonium in more nearly neutral or in alkaline solutions. Apparently the cranberry is capable of utilizing ammonium under slightly more acid conditions than are tomato and certain other plants. Of the reactions tried, glycine was most successful at pH 4.

6. Soil cultures indicate that in bog soil ammonium can be used at a somewhat higher hydrogen-ion concentration than in sand. The difference may be attributable to the greater buffer properties of the bog soil.

7. Tests indicate that in the glycine cultures of these experiments, glycine was absorbed as such.

8. Mycelium of the endophytic fungus was found throughout the stem tissues, especially in parenchyma and in the cells of embryonic tips. The

amount of mycelium appears to be directly correlated with the vigor of vegetative growth, being abundant in the growing tips of vigorous plants and less abundant and partially disintegrated in less vigorous plants.

9. Analysis of bog plants showed a correlation between degree of vegetative vigor and concentration of fats and lipoids in the leaves, the most vigorously vegetative plants having the lowest concentration of ether-soluble substances.

10. It is suggested that in the cranberry, as probably in other plants, there is little difference in histology and composition of elaborated materials between plants that have attained the same degree of vegetative vigor, although they may have been grown under different environmental conditions.

HORTICULTURAL DIVISION AND CRANBERRY SUBSTATION,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION,
NEW BRUNSWICK, N. J.

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RELATION OF WESTERN YELLOW PINE SEEDLINGS TO THE REACTION OF THE CULTURE SOLUTION

JOSEPH HOWELL, JR.

(WITH SEVEN FIGURES)

Introduction

The diversity of habitats under which western yellow pine (*Pinus ponderosa* Law.) grows throughout its extensive range indicates the ability of this species to adapt itself to many environmental conditions. **Sampson** (**34**) noted that the species grew best in acid soils and was stunted in alkaline soils, while **Baker** (**4**) found that soil reaction had little effect on the development of its seedlings. In the forests of the eastern United States **Wherry** (**41**) found that conifers had a predilection for the acid soils. **Baker** and **Korstian** (**5**) made an extensive study of the reaction of soils in the Great Basin, and found that the western yellow pine grew in soils of pH 7.0 to 9.0. The writer found that, in the Sierras, western yellow pine grows on soils of a reaction of pH 5.0 to 8.5. This gives a total known range of from pH 5.0 to 9.0, and, from all indications, does not cover the entire range of reaction over which the species can exist.

Accordingly, there were three objectives in this investigation, as follows: (1) To study the range of reaction (acidity and alkalinity) which the seedlings of western yellow pine can endure. (2) To observe the effects of the pH reaction on the growth and development of its seedlings. (3) To determine what effect the pH reaction has on the expressed sap of the seedlings.

Procedure

Investigators (**17, 37**) have found that soil and sand cultures are unreliable for studies of reaction, in that many other factors influence the results. The greater number of investigators (**7, 17, 18, 37**) have, therefore, resorted to water cultures, in which they were able to decrease the activity of many interfering factors. In this study water cultures were used in order to secure as nearly as possible an equality of conditions for plant growth. Table I lists the culture media used.

Two milliliters of 0.5 per cent. solution of ferric tartrate were added to the original culture solution, as well as a like portion each week. The molar solutions were made up separately, using C.P. salts and ordinary distilled water. The culture solution was made by diluting the required amounts of the molar solutions with ordinary distilled water. Care was taken in mixing the various solutions so as to prevent the precipitation of

TABLE I

COMPOSITION OF STOCK AND CULTURE SOLUTIONS

SALTS	MOLAR CONC. GRAMS PER LITER OF STOCK SOLUTION	CALIFORNIA SOLUTION; MOLAR SOLUTION PER LITER	IONS	P.P.M.	pH OF CULTURE
	gm.	*ml.*			
$Ca(NO_3)_2 \cdot 4H_2O$	236.2	3.9	Ca	157	5.2
			K	181	
KNO_3	101.1	3.6	Mg	55	
$MgSO_4 \cdot 7H_2O$	246.5	2.2	NO_3	709	
			SO_4	212	
KH_2PO_4	136.2	1.1	PO_4	105	

the phosphate ion. The ferric tartrate was added after the other solutions had been diluted and well mixed in order to prevent the precipitation of insoluble iron compounds.

Changes of solution were made every four weeks in order to prevent serious changes in the composition of the cultures. The pH values of the cultures were adjusted by the addition of measured quantities of standard sulphuric acid or of potassium hydroxide. The pH values were determined colorimetrically (**8**), with frequent checks with the hydrogen electrode. Examinations and adjustments were made daily because of the rapid changes in the reaction on the alkaline side of neutrality.

TABLE II

MAXIMUM CHANGES OF REACTION IN 24 HOURS

pH OF SOLUTION	MAXIMUM CHANGE	pH OF SOLUTION	MAXIMUM CHANGE
2.7	0.1	7.0	0.3
3.0	0.1	8.0	0.3
4.0	0.2	9.0	0.4
4.5	0.2	10.0	0.4
5.0	0.2	11.0	0.5
6.0	0.2		

Selected seeds of western yellow pine collected near Georgetown, California, germinated between sterile blotters, and selected germinated seeds were transferred to the water cultures. Stoneware jars of 14-liters' capacity were used as containers, and two such jars containing 35 seedlings constituted a set. The following pH values were used: pH 2.7, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0. The plants were allowed to grow for a period of 133 days, at the end of which time they were harvested and subjected to measurement and analysis. The plant material was frozen prior to the physico-chemical and chemical measurements.

In previous experiments, seedling variation had been found to be great, the probable error varying from ± 15 to ± 45 per cent. of the means, de-

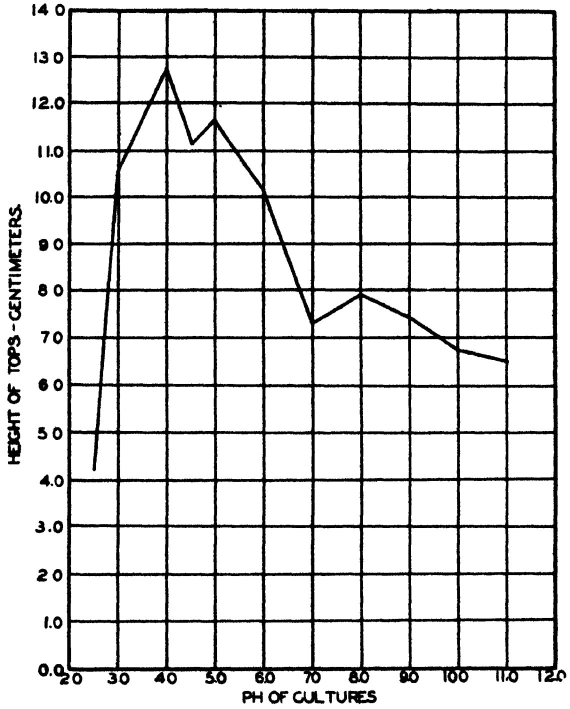

FIG. 1. Effect of reaction on height growth.

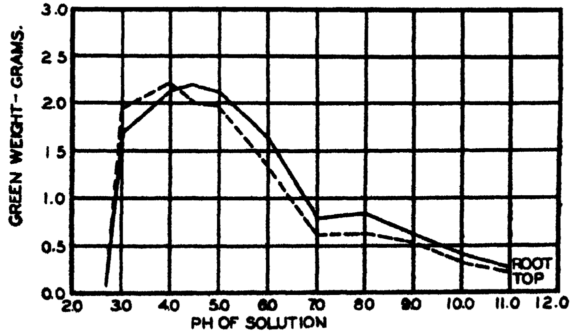

FIG. 2. Curves showing effects of reaction on seedlings of western yellow pine.

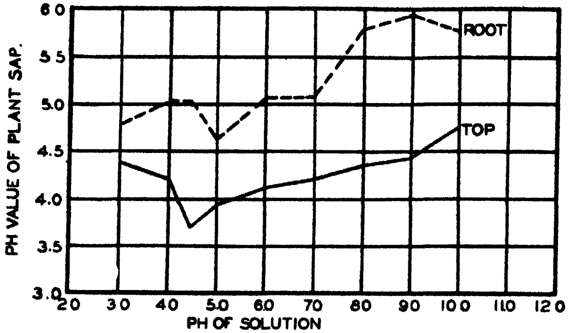

FIG. 3. Curves showing effects of reaction on the pH values of the plant sap.

pending on the measure. The measures for height growth had the smallest probable error; those for the green weights and dry weights showed the largest probable error.

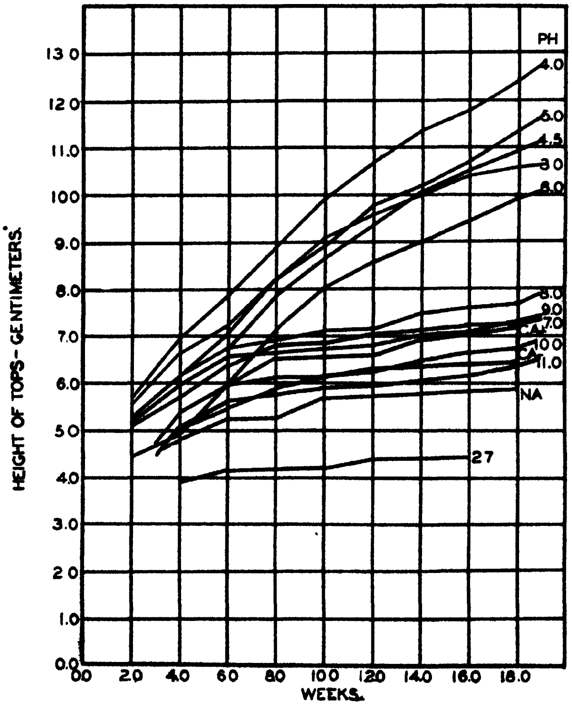

FIG. 4. March of height growth for seedlings; each curve indicates a definite pH reaction.

Results

In this experiment it was found that seedlings of western yellow pine would exist over an extensive range of reaction (figs. 1, 2, 3, 4). The growth limit on the acid side was at pH 2.7, but the growth limit was unde-

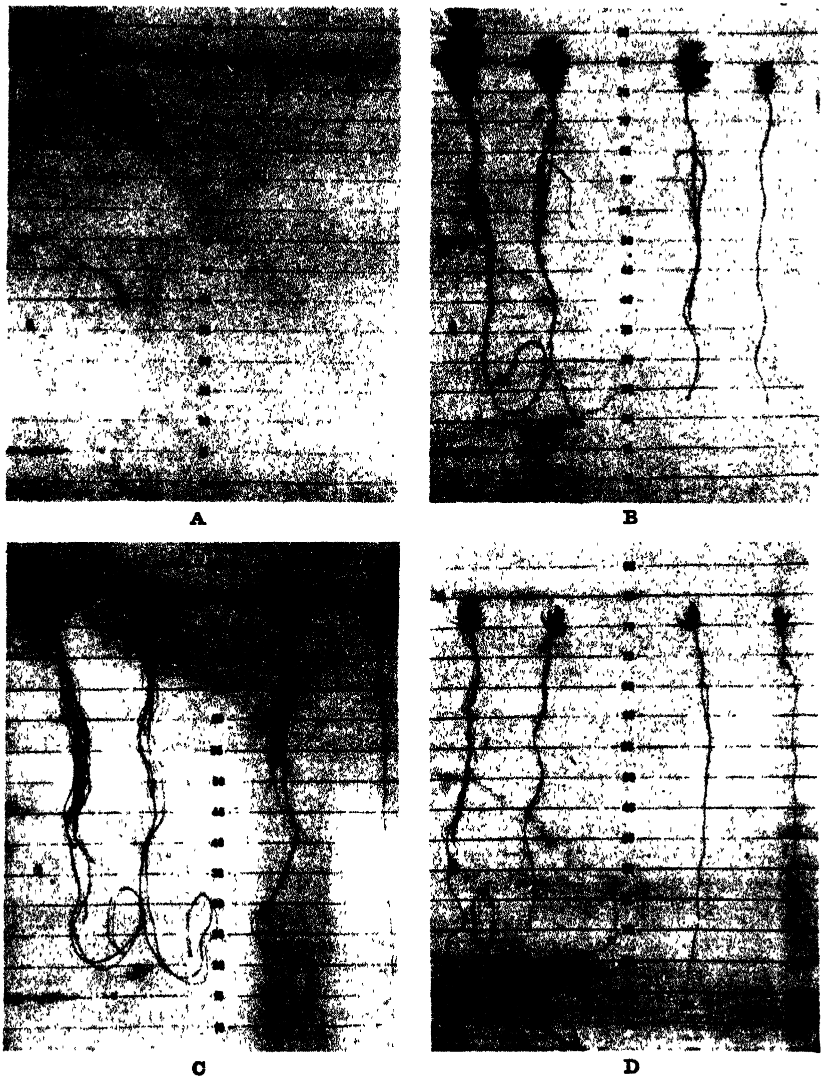

Fig. 5. Western yellow pine seedlings grown in culture ranging in pH from 2.7 to 7.0: *A*, pH 2.7; *B*, pH 4.0; *C*, pH 5.0; *D*, pH 7.0.

termined for the alkaline range. However, a few plants existed in cultures of pH 11.0 for the term of the experiment, and this may be taken as the limit of growth for the alkaline range. This gives a total range of reaction of from pH 2.7 to pH 11.0.

The general appearance of the plants growing in the acid solutions was striking when contrasted with those grown in the alkaline cultures (figs. 5, 6). The seedlings in the acid solutions were vigorous and well developed while those in the alkaline cultures were stunted and chlorotic.

Physical measurements of the seedlings were taken at the termination of the growth period and are presented in table III.

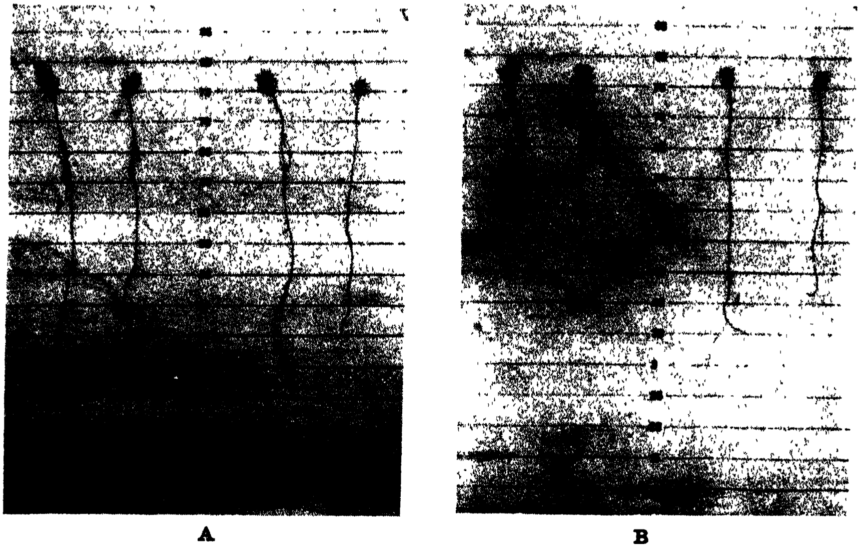
A B

FIG. 6. Western yellow pine seedlings grown in culture solutions of 9.0 and 10.0: *A*, pH 9.0; *B*, pH 10.0.

Height growth measurements were taken at intervals of seven days and present an interesting set of growth curves (fig. 4). Three distinct bands are formed; namely, the acid band, the alkaline band, and the single curve for the acid growth limit. These curves give ample evidence as to the efficacy of the acid cultures. The growth rate of the plants was little diminished at the termination of the experiment, whereas in the alkaline cultures the rate of growth was greatly retarded. The plants growing in the very acid culture (pH 2.7) had a negligible growth rate.

The length of the tap root was affected by the reaction of the culture. Those in the acid media appeared healthy and vigorous, whereas those in the alkaline solutions were brown and thickened, with a tendency to disin-

TABLE III

SUMMARY OF PHYSICAL AND CHEMICAL MEASUREMENTS OF SEEDLINGS

MEASUREMENTS	pH 2.7*		pH 3.0		pH 4.0		pH 4.5		pH 5.0		pH 6.0
	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS
1. Number of plants	7	7	29	29	33	33	32	32	32	32	34
2. Height of tops (cm.)	4.400	—	10.6000	—	12.7600	—	11.0400	—	11.6200	—	10.1200
3. Length of roots (cm.)	—	6.870	—	50.8000	—	65.6000	—	69.4000	—	78.3000	—
4. No. of lateral roots	—	6	—	72.6000	—	85.0000	—	87.7000	—	92.1000	—
5. Diameter of stem (mm.)	0.940	—	2.1000	—	2.4300	—	2.2900	—	2.4100	—	2.2800
6. Green weight (gm.)	0.097	0.054	1.6900	1.9200	2.1500	2.2000	2.1600	2.0000	2.0900	1.9500	1.6100
7. Top-root ratio	1.790	—	0.8800	—	0.9800	—	1.0800	—	1.0700	—	1.2000
8. Yield of sap (ml.)	—	—	25.0000	37.8000	52.0000	34.5000	41.0000	30.5000	38.5000	27.5000	28.5000
9. Weight of 1 ml. of sap	—	—	1.0130	1.0080	1.0230	1.0080	1.0300	1.0040	1.0350	1.0060	1.0350
10. Index of refraction	1.356	—	1.3440	1.3380	1.3450	1.3390	1.3420	1.3380	1.3460	1.3390	1.3490
11. Percentage of solids	15.100	—	7.5000	3.2000	8.3000	4.4000	6.4000	3.3000	8.7000	4.1000	10.5000
12. Weight of solids (gm.)	—	—	0.0760	0.0320	0.0850	0.0440	0.0660	0.0330	0.0900	0.0410	0.1080
13. Freezing-point depression	—	—	0.9340	0.4440	0.9960	0.5770	0.7770	0.4520	0.9570	0.6030	1.0500
14. Osmotic pressure (atmos.)	—	—	11.2500	5.3400	11.9900	6.9500	15.4300	10.2000	11.5200	7.2600	12.6400
15. Mean molecular weights	—	—	163.1000	139.4000	181.5000	152.1000	165.8000	141.6000	187.2000	133.4000	206.7000
16. Specific conductivity (k)	—	—	0.0007	0.0005	0.0008	0.0007	0.0006	0.0006	0.0007	0.0007	0.0012
17. Saponification number	—	—	14.2200	8.8900	15.1500	9.7200	15.4300	10.2000	17.5000	8.5600	19.5200
18. Acid number	—	—	6.0200	4.8400	7.0100	6.0500	5.2300	3.7800	7.1900	4.5700	8.7500
19. Ether number	—	—	7.2000	4.0500	8.1400	3.6700	10.2000	6.4200	10.3100	4.0100	10.7700
20. Iodine number	—	—	1.3300	0.6600	2.1200	0.5500	1.3000	0.5100	1.5400	0.9000	1.7300
21. pH value of sap	—	—	4.3900	4.7800	4.2200	5.0300	3.7000	5.0100	3.9200	4.5500	4.1200
22. N/10 NaOH (ml.) to neutralize 5 ml. sap†	—	—	3.3400	2.0600	3.7000	2.2000	3.4500	1.8100	4.3700	2.2600	3.7500
23. N acid (ml.) neutralized per plant	—	—	—	0.7100	—	0.6500	—	0.5700	—	0.7300	—
24. N alkali (ml.) neutralized per plant	—	1.410	—	—	—	—	—	—	—	—	—

* Insufficient material for examination.

† Point of neutralization taken as pH 8.3.

TABLE III (*Continued*)

Measurements	pH 6.0	pH 7.0		pH 8.0		pH 9.0		pH 10.0		pH.11.0	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
1. Number of plants	34	18	18	19	19	21	21	8	8	2	2
2. Height of tops (cm.)	——	7.2300	——	7.8900	——	7.4000	——	6.8500	——	6.500	——
3. Length of roots (cm.)	89.700	——	57.8000	——	56.8000	——	46.8000	——	29.6000	——	31.700
4. No. of lateral roots	89.700	——	57.2000	——	55.2000	——	51.0000	——	31.9000	——	36.000
5. Diameter of stem (mm.)	——	1.7800	——	1.9300	——	1.6600	——	1.3600	——	1.500	——
6. Green weight (gm.)	1.340	0.7500	0.6000	0.8600	0.6200	0.5900	0.5100	0.3900	0.2900	0.240	0.200
7. Top-root ratio	——	1.2500	——	1.3900	——	1.1600	——	1.3200	——	1.200	——
8. Yield of sap (ml.)	21.500	9.0000	7.5000	10.0000	10.0000	6.0000	7.0000	5.0000	4.0000	%	%
9. Weight of 1 ml. of sap	1.015	1.0140	0.9960	1.0290	0.9780	1.0140	0.9630	1.0130	0.9720	%	%
10. Index of refraction	1.339	1.3460	1.3390	1.3490	1.3370	1.3450	1.3370	1.3510	1.3390	1.351	1.338
11. Percentage of solids	4.500	9.0000	4.3000	10.8000	2.8000	8.2000	2.8000	11.9000	3.7000	11.800	3.500
12. Weight of solids (gm.)	0.056	0.0910	0.0430	0.1110	0.0270	0.0840	0.0270	0.1210	0.0360	%	%
13. Freezing-point depression	0.678	0.8050	0.4650	1.1080	0.3240	0.8530	0.3770	0.6980	0.3540	%	%
14. Osmotic pressure (atmos.)	8.170	9.6900	5.6000	13.3400	3.9100	12.2600	4.5400	8.1400	4.2700	%	%
15. Mean molecular weights	128.400	231.1000	187.3000	204.0000	166.7000	199.7000	143.9000	226.6000	198.0000	%	%
16. Specific conductivity (k)	——	0.0009	0.0008	0.0013	0.0008	0.0013	.0008	0.0006	.0003	%	%
17. Saponification number	8.580	15.1000	6.7000	16.3000	4.3000	14.0000	5.2000	5.6000	2.2000	%	%
18. Acid number	5.510	8.2000	4.5000	8.4000	3.1000	6.6000	2.3000	4.6000	1.9000	%	%
19. Ether number	3.070	6.9000	2.2000	7.9000	0.9000	7.4000	2.9000	1.0000	0.3000	%	%
20. Iodine number	1.170	2.1000	0.7500	1.6500	0.3300	1.8200	0.3400	——	0.2000	%	%
21. pH value of sap	5.050	4.2100	5.1200	4.3600	5.7700	4.4100	5.9700	4.7400	5.7300	%	%
22. N/10 NaOH (ml.) to neutralize 5 ml. sap†	2.000	3.9000	1.0000	4.4000	1.7000	2.8000	0.8000	1.9500	0.8500	%	%
23. N acid (ml.) neutralized per plant	0.290	——	——	——	——	——	——	——	——	——	——
24. N alkali (ml.) neutralized per plant	——	——	0.1000	——	3.4200	——	7.9900	——	18.6900	——	21.580

* Insufficient material for examination.

† Point of neutralization taken as pH 8.3.

% Sap mixed with that from the plants grown at pH 10.0.

tegrate. The roots of the plants growing in the cultures of pH 2.7 were thickened and brittle. The number of lateral roots followed the same trend as the length of tap roots, and therefore it is reasonable to assume that they were dependent on this factor.

The dry weights of plants have been considered as criteria for metabolism, but in this case it was not possible to obtain the dry weights. The green weights followed the trend of the height of tops, exhibiting the usual points of weakness. It was evident that the reaction of the culture medium influenced growth and development of the seedlings.

Immediately after harvesting, the plant material was frozen and, for 48 hours, held at a temperature of – 20° C. The frozen tissue was quickly thawed in hot water and the sap expressed in a small hand-press. The expressed sap, refrozen, was preserved for future studies.

The pH value of the mixed sap was determined by the hydrogen electrode (**8**), at the same time that the titration curves were made (fig. 7).

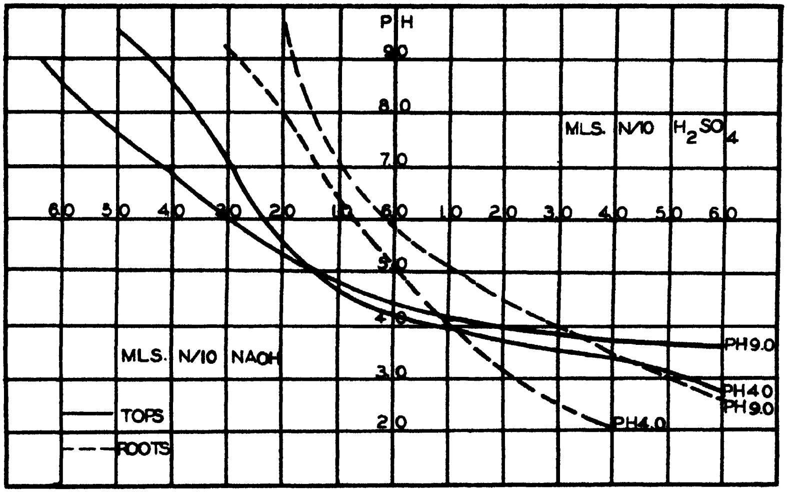

FIG. 7. Titration curves for the plant sap from the tops and roots of plants grown in the cultures at pH 4.0 and 9.0.

The pH values for the sap from the tops tended to vary from the acid to the alkaline side; however, the values decreased at pH 4.0, 4.5, and 5.0. The values for the sap from the roots followed the same trend as did those for the tops. In all cases the sap was found to be distinctly acid.

The titration curves for the sap, both of the tops and the roots, form definite and strong buffer curves. The sap from the roots had distinctly less buffer action than the sap from the tops. These results showed that the sap of the roots was more dilute or contained less material that was capable of acting as a buffer.

The freezing-point depressions were taken with a Haidenhain thermometer, standardized with freshly boiled distilled water. The resulting data showed that the sap was affected directly or indirectly by the reaction of the cultures.

The saponification number, acid number, and iodine number, all of which were found by using standard methods, varied and usually showed differences in the general trend at pH 4.5 and 7.0. The constituents of the sap possibly were altered as to quantity and quality. This alteration in the plant sap presumably affected the endurance of the plants toward the effects of reaction. The physiology of this point will be discussed in another section.

Other measurements were made, the results of which are given in table III.

A change in the pH values of the cultures was noted, with the result that the plants were found to influence the specific reaction. Plants are known to absorb ions (**1, 2, 7, 19, 21, 22, 23, 24, 29, 31, 36, 37**) from solution and thereby change its reaction. Also, the plant may excrete ions (**19, 25**) and in that way alter the pH value. Upon examination of the solutions for a decrease in the ions, it was found that absorption was negligible and could not cause any great change in reaction (table IV). The excretion of carbon dioxide was next studied in some detail, but the results were again negligible. No further examinations were made, but it was assumed that some organic substances were excreted by the seedlings, causing changes in the reaction. The magnitude of the change of reaction depends upon the buffer action of the solution. The potassium acid phosphate was the buffer agent in the solution, but this was precipitated in the alkaline solutions as insoluble calcium phosphate.

TABLE IV

ABSORPTION OF IONS BY THE SEEDLINGS

ION	MILLI-EQUIVALENTS*			
	FIRST SET	SECOND SET	THIRD SET	MEAN
Ca	0.180	0.095	0.075	0.117
PO_4	0.270	0.111	0.084	0.155
NO_3	0.166	0.055	0.056	0.092

* One hundred plants for 10 hours.

There was little doubt that the plants were instrumental in changing the reaction of the cultures in some manner, such as through the absorption or excretion of ions or molecules.

Certain investigators (**5, 26**) have stated that western yellow pine fails to grow when large quantities of lime are present in the soil. To determine the accuracy of this statement, one set of plants was grown in a culture containing no calcium; another in a culture containing an average of 1000 p.p.m. of calcium; and a third in a culture containing an adequate amount of calcium, with the alkalinity produced by the addition of sodium hydroxide. The solutions were kept at pH 9.0. The results were not greatly different in all cases, but the plants growing in the solutions containing an excess of calcium were superior to the other two. The sodium-treated plants were inferior to the others. The plants, according to this evidence, withstood large quantities of calcium; they also could exist in a solution deficient in calcium.

The leaves of the seedlings in the alkaline solutions were yellowish in color, and it was assumed that the plants could not assimilate and translocate sufficient iron (**37**). The metabolism of the plants seems to be disturbed in the alkaline media, thus producing a poor color in the leaves. Also, iron has been found to become insoluble after having been in solution for a reasonable time.

Discussion

Several investigators (**2, 28, 30, 33, 35, 41**) have casually studied the effects of soil reaction on forest trees, although little work has been carried on in detail. The opinion of European investigators is that the reaction of the soil is a potent factor in forest planting, natural regeneration, and forest production. The conifers were observed to prefer an acid reaction for their best growth, and soils under coniferous forests were found to be on the acid side of neutrality. Conifers did not do well on the alkaline soils, but certain tolerant species withstood an alkaline condition and competed with more resistant plants.

The concentration of the soil solution (**1, 2, 18, 20, 21, 22, 24, 39**) is an important factor in plant growth. Also there must be considered the concentration of each ion, since HOAGLAND (**19, 21**) and others have found that there is a minimum quantity which limits maximum growth. Small variations in the concentration do not appreciably affect the growth of the plants. The concentration of the soil solution also influences the concentration of the sap (**23**); it affects certain ions (**36**), and in turn alters the buffer system (**24, 29**).

The hydrogen-ion concentration, having been studied in reference to agricultural plants (**2, 40**), has been found to influence the rate of growth. A greater number of plants prefer a slightly acid medium for their best growth. The seedlings of western yellow pine prefer an even greater degree of acidity than do most cultivated plants. The absorption of nitrates

(**20**) has been found to be greater in the acid solutions, and would, therefore, increase the rate of growth of the seedlings.

Consequently, the behavior of western yellow pine seedlings in prefering acid soils is not different from that of other plants, the only difference being that they prefer a more acid medium. As found by WHERRY (**41**), most conifers prefer an acid soil rather than a neutral or alkaline one.

The pH value of the various plant tissues is characteristic (**3, 12, 13, 32**); the cell sap is usually acid and the protoplasm alkaline. The mixed sap for the western yellow pine seedlings was decidedly acid. However, no examination was made of the various tissues. The mixed saps were well buffered, as shown by the titration curves, although slight differences are to be noted. The buffer system of a plant may be affected by the external media, according to THERON (**37**) and others (**14, 29**), and this system is affected by the acid and alkaline reserves in the plant. Some investigators have concluded that certain specific substances are responsible for the buffer system. In the expressed sap of the plants used in this experiment, large quantities of catechol tannins were found, as well as other resinous materials.

The seedlings growing in the alkaline cultures were chlorotic and in some cases the yellow color was pronounced. Chlorosis has been attributed to many sources (**11**), but it was evident in this experiment that a lack of iron in the leaves was responsible. The plants either did not assimilate the element properly or failed to translocate it after assimilation. The kind of iron compound (**9, 11, 38**) and the type of iron have great influence on the ability of the iron to remain in solution. Even with the best of care it was not possible to relieve the situation. This appearance of the seedlings can be used as an indicator of adverse alkaline soils.

As has previously been stated, the plants were capable of changing the pH value of the culture medium, although the change was usually slight, except in the alkaline cultures. By what process the seedlings changed the reaction of the solutions remains unknown. Their ability to bring about this change does not mean that the plants would effect any great change in so well buffered a medium as the soil.

The constituents of the sap of the plants determine to some extent their ability to withstand environmental factors. Plants with a sap that is highly buffered will usually withstand a wide range of conditions. There are in evidence points which investigators (**2, 16**) have designated as isoelectric. In this case these points are at pH 4.5 and 7.0, and they were noticeably inferior to those in the cultures of lower and higher pH values. Plants subject to these conditions would therefore be inferior and less likely to withstand competition.

The hydrogen ion (**7, 10, 27**) has been considered more toxic than the hydroxyl ion, but it has been shown in this experiment that the reverse is true. Since such is the case in this study, the best forests should be in the regions of slightly acid soils, and the seedlings produced in alkaline materials would be inferior in form and color.

Hesselman (**15**) concludes that soil acidity is closely related to the form of available nitrogen. In this case then the plants should assimilate nitrogen in larger quantities in the acid solutions; and since the quantity of nitrogen used by western yellow pine seedlings is small, there should be little danger of competition.

Summary

1. Western yellow pine seedlings prefer an acid medium for their best growth.
2. Under field conditions other factors may have more influence on growth than the soil reaction.
3. The osmotic pressure of the sap indicates that soil reaction affects the seedlings as well as do certain other factors.
4. The buffer system of the seedlings is dependent on the organic materials found in the sap as well as on the inorganic substances.
5. The ability of the seedlings to change reaction is not to be construed as an effective measure in reducing soil alkalinity. Débris from the forest cover may be more effective because of the relatively high resin content.
6. Calcium is not deleterious to the seedlings. The fact that the plant does not assimilate the ion in alkaline soils may be of more importance.
7. The seedlings withstand a diversity of conditions, especially in regard to soil acidity and alkalinity.

University of California,
Berkeley, California.

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ABSORPTION OF OXYGEN BY ROOTS WHEN THE SHOOT IS IN DARKNESS OR IN LIGHT

W. A. CANNON

The writer has observed a variation in the oxygen content of the substratum of roots which appears to be related to certain of the environmental conditions of the shoot, especially to light and darkness, to temperature and to the relative humidity, or to the rate of evaporation. It had been observed previously, also, that there might be a direct relation, at least in time, between root growth and the lighting conditions of the shoot,[1] although in the preliminary experiments here referred to no relation was found between the rate of root growth and the moisture condition of the air. The present paper gives an account of experiments on the possible relation of the shoot to light and darkness and the variation of the oxygen content of the root environment.

As to the results, it can be premised that they suggest the probability that roots may obtain the oxygen used by them from at least two sources, namely, (1) from their immediate environment and (or) (2) from the shoot. In the latter event it is probably to be regarded as a product of photosynthesis. Thus, in the root, and also in the plant as a whole, autoaeration may in part obtain. Oxygen presumably derived from photosynthesis has, for convenience, been referred to as "internal" oxygen[2] and may be an important feature in the aeration of the tissues of all green plants.

Experiments

The experiments which are summarized in this paper were carried out[3] in March–September, 1931 and 1932, at Stanford University. Although in the course of the work several species were used, the number was finally reduced to two, namely, a willow (*Salix laevigata* Bebb) and the sunflower (*Helianthus annuus* L.). The former occurs along water courses on the campus of the University and the latter was obtained in a local market; the variety is not known.

Water cultures were used. The plants were grown in Knop's solution but were transferred for tests to distilled water. This was done because in the Winkler method, which was used, nitrites interfere with determination of oxygen; and when Knop's solution stands for some time, nitrites appear in sufficient amount to invalidate the determinations.

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[1] CANNON, W. A. Year Book, Carnegie Inst. Washington, no. 24, 1925; no. 30, 1930; Physiological features of roots, Pub. no. 368, Carnegie Inst. Washington; and On the variation of the oxygen content of culture solutions. Science 75: 108–109. 1932.

[2] BEARDSLEY, G. F., and CANNON, W. A. Note on the effects of a mud-flow at Mt. Shasta on the vegetation. Ecology 11: 326–336. 1930.

[3] The study was carried on at Stanford University with assistance of grants from the American Association for the Advancement of Science, in 1931, and the National Research Council, 1932.

The plants were kept in one-quart fruit jars which were made light-tight and which were well sealed. Each jar carried a thermometer, inlet and outlet tubes, and one or more plants. The tubes were closed with a short piece of rubber tubing and a pinchcock or clamp. The most satisfactory inlet tube was a small calcium chloride drying tube which was provided with a rubber stopper with one hole carrying a capillary tube. This arrangement accomplished two ends; it permitted the detection of defective sealing, and provided water to replace that taken up by the plant. When it was desired to have a solution with no oxygen (or very little) at the beginning of any test, this was accomplished by utilizing the solution in which a plant, such as a willow, had been growing in darkness for a period of 12 hours or more. By appropriate arrangement of the cultures it was possible to withdraw the water and run it into experimental jars without appreciable admixture of oxygen from atmospheric air.

In general the method of conducting the experiments was to keep the plants under observation in darkness or in light for equal periods, usually from 1.5 to 3 hours. During the light period, preferably at midday, the plants were directly exposed to sunlight; or they were shaded lightly by wire-mesh screens; or they were covered with a bell glass which was placed either in direct sunlight or under the screens. When bell glasses were used, cooling was effected by running water. The results from artificial lighting were entirely negative, and no report is given of such experiments.

The Thompson-Miller apparatus for the micro-determination of dissolved oxygen by Winkler's method was used.[4] The only change in the apparatus found advisable was the introduction of a glass bead into the sample chamber to assist in the mixing of the ingredients.

The sample chamber had a capacity of about 5 cc. but it usually varied very considerably so that calibration was necessary.

In tables I and II are given the most important data derived from the study of willow and sunflower. The willows were cuttings which had been rooted and were kept in a nutrient solution except during actual tests. The sunflower seedlings were treated similarly.

[4] Ind. & Eng. Chem. 1928. See also SNOKE, A. W. The determination of dissolved oxygen with the micro-Winkler apparatus of Thompson and Miller. Ecology 10: 163–164. 1929. A further account is by ZIMMERMAN, Rept. and Proc. IXth International Congress, London, 7th to 15th August, 1930. For the general method see SUTTON's Volumetric analysis, p. 480. 1924; Standard methods of the examination of water and sewage, 2d ed., 1912, American Public Health Assoc.; and the critique by ALSTERBERG, G. Die Winklersche Bestimmungsmethode für in Wasser gelösten, elementaren Sauerstoff sovie ihre Anwendung bei Anwesenheit oxydierbarer Substanzen. Biochem. Zeitschr. 170: 30–75. 1926.

TABLE I

AMOUNT OF OXYGEN REMOVED BY ROOTS FROM DISTILLED WATER WHEN THE SHOOT HAD BEEN IN DARKNESS OR IN SUNLIGHT FOR GIVEN PERIODS OF TIME

I. *Helianthus annuus**

EXP. NO.	TEMPERATURE			TIME PERIOD	OXYGEN CONSUMPTION PER LITER	
	CONTROL	CULTURE	AIR		DARKNESS	SUNLIGHT
	°C.	°C.	°C.		mg.	mg.
1	21.5	24.5	25.5	8 to 10 A. M.		2.94
	21.5	23.0	22.5	11 A. M. to 2 P. M.	1.91	
	24.0	24.5	24.0	2 P. M. to 5 P. M.		2.40
2	21.5	22.0	22.5	8 to 11 A. M.	4.40	
	21.5	25.5	30.0	11 A. M. to 2 P. M.		0.00
	24.0	23.0	23.2	2 to 5 P. M.	2.56	
3	19.5	24.5	25.0	8 to 11 A. M.		2.80
	21.0	23.0	23.0	11 A. M. to 2 P. M.	1.68	
	25.0	25.0	23.5	2 to 5 P. M.		0.40
4	19.5	21.5	22.0	8 to 11 A. M.	2.06	
	21.0	25.0	31.0	11 A. M. to 2 P. M.		2.36
	25.0	23.5	25.0	2 to 5 P. M.	1.41	
5	19.0	22.0	22.0	8 to 11 A. M.	0.84	
	23.0	24.0	23.0	11 A. M. to 2 P. M.		2.23
	26.0	24.0	23.0	2 to 5 P. M.	2.55	
6	19.0	22.5	22.0	8 to 11 A. M.		2.75
	23.0	23.0	23.0	11 A. M. to 2 P. M.	2.20	
	26.0	23.5	22.5	2 to 5 P. M.		3.02
7	18.0	19.5	27.0	8 to 10 A. M.	0.17	
	18.5	21.0	24.0	10 A. M. to 12 M.		0.00
	21.5	21.0	24.0	12 M. to 2 P. M.	0.40	
	23.0	21.0	24.0	2 to 4 P. M.		1.29
	23.5	21.5	18.5	4 to 6 P. M.	0.49	
8	18.0	21.5	20.0	8 to 10 A. M.		0.39
	18.5	20.5	20.0	10 A. M. to 12 M.	0.88	
	21.5	21.5	24.0	12 M. to 2 P. M.		0.00
	23.0	22.0	21.0	2 to 4 P. M.	2.64	
	23.5	20.5	24.0	4 to 6 P. M.		3.14
9	18.5	21.0	22.5	8 to 11 A. M.	3.47	
	20.0	26.0	24.0	11 A. M. to 1 P. M.		1.91
10	18.5	21.0	22.5	8 to 11 A. M.	2.71	
	20.0	25.0	24.0	11 A. M. to 2 P. M.		1.15
11	16.0	20.5	21.5	8 to 11 A. M.	3.58	
	18.0	24.5	24.0	11 A. M. to 2 P. M.		2.35
12	16.0	20.5	21.5	8 to 11 A. M.	2.37	
	18.0	24.5	24.0	11 A. M. to 2 P. M.		1.36

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* The shoot was kept under a bell glass when exposed to the light and also either under a bell glass or under a tight cover when in the dark. In any case the atmosphere about the shoot was saturated, or nearly so.

TABLE I—(*Continued*)

Exp. no.	Temperature			Time period	Oxygen consumption per liter	
	Control	Culture	Air		Darkness	Sunlight
	°C.	°C.	°C.		mg.	mg.
13	15.0	18.5	21.5	8 to 11 A. M.	4.39	
	18.0	24.0	24.0	11 A. M. to 2 P. M.	……	1.86
14	15.0	18.5	21.5	8 to 11 A. M.	1.87	
	18.0	24.0	24.0	11 A. M. to 2 P. M.		0.00
15	16.5	19.0	19.0	8 to 11 A. M.	2.53	
	17.0	23.0	20.5	11 A. M. to 2 P. M.		1.19
16	16.5	19.0	19.0	8 to 11 A. M.	1.92	
	17.0	23.0	20.5	11 A. M. to 2 P. M.		1.64
17	11.5	17.0	20.0	8 to 11 A. M.	4.18	
	13.5	24.0	21.0	11 A. M. to 2 P. M.		1.24
18	11.5	17.0	20.0	8 to 11 A. M.	2.27	
	13.5	19.5	21.0	11 A. M. to 2 P. M.		1.37
19	14.0	17.5	20.0	8 to 11 A. M.	2.83	
	15.0	22.0	15.0	11 A. M. to 2 P. M.		2.53
20	14.0	17.5	20.0	8 to 11 A. M.	1.62	
	15.0	22.5	22.0	11 A. M. to 2 P. M.		0.72
21	17.0	18.0	19.5	8 to 11 A. M.	2.93	
	17.0	23.0	24.0	11 A. M. to 2 P. M.		4.71
22	18.0	19.5	25.0	8 to 11 A. M.	3.03	
	18.0	23.0	24.0	11 A. M. to 2 P. M.		2.90
23	18.0	19.5	25.0	8 to 11 A. M.	2.49	
	18.0	24.0	24.5	11 A. M. to 2 P. M.		2.70
24	18.0	19.5	25.0	8 to 11 A. M.	2.76	
	18.0	24.0	24.5	11 A. M. to 2 P. M.		1.89
25	23.8	23.5	26.5	8 to 10:30 A. M.	5.60	
	20.5	20.0	26.5	11:30 A. M. to 2 P. M.		6.27
26	18.8	23.0	23.0	8 to 9:30 A. M.	4.53	
	21.7	23.0	23.9	9:30 to 11 A. M.		4.02
	23.5	23.5	25.0	12:55 to 2:25 P. M		4.78

Discussion

This study is concerned with the rate at which roots absorb dissolved oxygen when the shoot is either in darkness or in light, especially in direct sunlight. Twenty-six experiments were carried out on the sunflower and twenty-seven on rooted willow cuttings. Of the 53 experiments summarized in tables I and II, 60 per cent. showed a slower rate of oxygen consumption by the roots when the shoot was in sunlight. In the sunflower the rate was

TABLE II

OXYGEN REMOVED BY ROOTS FROM DISTILLED WATER WHEN THE SHOOT HAD BEEN IN DARKNESS OR IN SUNLIGHT FOR GIVEN PERIODS OF TIME

II. *Salix laevigata*

EXP. NO.	TEMPERATURE			TIME PERIOD	OXYGEN CONSUMPTION PER LITER	
	CONTROL	SOLUTION	AIR		DARKNESS	LIGHT
	°C.	°C.	°C.		mg.	mg.
27	23.4	23.0		8 to 11 A. M.	4.12	
	22.2	22.0		11 A. M. to 2 P. M.		3.75*
28	24.0	24.0		8 to 11 A. M.	4.14	
	24.8	24.5	22.2	11 A. M. to 2 P. M.		5.32*
29	24.0	24.0		8 to 11 A. M.	3.26	
	24.0	24.0		11 A. M. to 2 P. M.		1.71*
30	24.6	22.5		8 to 11 A. M.	3.18	
	21.5	21.0		11 A. M. to 2 P. M.		2.66*
31	22.0	22.0		8 to 11 A. M.	5.90	
	23.0	22.0		11 A. M. to 2 P. M.		4.70*
32	23.5	22.5		8 to 11 A. M.	4.40	
	22.7	23.0		11 A. M. to 2 P. M.		5.60*
33	23.0	22.5		8 to 11 A. M.	3.82	
	26.0	24.0		11 A. M. to 2 P. M.		4.42*
34	22.8	23.0		8 to 11 A. M.	1.19	
	23.7	24.0		11 A. M. to 2 P. M.		1.12*
35	22.5	22.0	24.5	8 to 11 A. M.	6.93	
	22.0	22.5	23.9	11 A. M. to 2 P. M.		7.38*
36	23.0	22.5	22.5	8 to 9: 30 A. M.	6.04	
	22.5	23.0		9: 30 to 11 A. M.		5.20*
	23.3	23.5	26.7	12: 30 to 2 P. M.		5.44*
37	22.7	22.0	22.0	8 to 9: 30 A. M.	5.32	
	22.8	23.0		9: 30 to 11 A. M.		5.44*
	22.0	22.0	25.0	1 to 2: 30 P. M.		4.48*
38	23.4	23.4	23.4	8 to 10 A. M.	5.62	
	23.0	24.4		10 A. M. to 12 M.		5.12*
	24.2	22.5		12 M. to 2 P. M.		5.58*
	23.3	22.5		2 to 4 P. M.		5.96*
39	21.5	21.0	21.0	8 to 9: 30 A. M.	4.42	
	20.0	21.0	22.2	9: 30 to 11 A. M.		3.43*
	21.6	21.5	23.3	1 to 2: 30 P. M.		4.00*
40	20.8	20.0	23.0	9 to 10: 30 A. M.	3.24	
	21.0	21.0	17.2	10: 30 A. M. to 12 M.		5.30*
41	21.5	21.5	21.5	8 to 9: 30 A. M.	4.25	
	20.5	21.5	23.9	9: 30 to 11 A. M.		5.22[b]
	22.7	26.0	24.4	12: 30 to 2 P. M.		5.52

TABLE II—(*Continued*)

EXP. NO.	TEMPERATURE			TIME PERIOD	OXYGEN CONSUMPTION PER LITER	
	CONTROL	SOLUTION	AIR		DARKNESS	LIGHT
	°*C.*	°*C.*	°*C.*		*mg.*	*mg.*
42	22.2	22.5		8 to 9:30 A. M.	3.58	
	22.8	21.5	23.3	9:30 to 11 A. M.		3.29[b]
43	22.5	22.0		8 to 11 A. M.	5.82	
	21.5	22.0	23.3	11 A. M. to 2 P. M.		5.25[b]
44	22.3	22.0		8 to 11 A. M.	5.89	
	22.0	22.0	24.4	11 A. M. to 2 P. M.		5.11[b]
45	20.5	20.5	20.5	8 to 11 A. M.	5.30	
	18.1	20.5	22.2	11 A. M. to 2 P. M.		5.18[b]
46	20.3	20.0	23.0	8 to 11 A. M.	5.92	
	19.1	21.5	25.0	11 A. M. to 2 P. M.		6.61
47	20.5	20.5	22.5	8 to 11 A. M.	6.71	
	19.1	20.0	23.3	11 A. M. to 2 P. M.		6.24[b]
48	20.3	20.0	23.0	8 to 11 A. M.	5.94	
	22.3	21.0	20.6	11 A. M. to 2 P. M.		5.79*
49	20.5	20.0	22.7	8 to 11 A. M.	6.43	
	22.3	21.0	28.9	11 A. M. to 2 P. M.		6.96*
50	20.8	20.2	23.0	8 to 11 A. M.	5.90	
	22.9	24.0	23.3	11 A. M. to 2 P. M.		6.50[b]
51	20.7	20.4		8 to 11 A. M.	7.09	
	24.0	25.5	27.6	11 A. M. to 2 P. M.		6.40[b]
52	24.2	24.0	24.5	8 to 11 A. M.	7.14	
	22.0	22.0	24.4	11 A. M. to 2 P. M.		6.79[b]
53	23.3	23.0	22.0	8 to 9:30 A. M.	5.30	
	19.0	22.0	25.6	9:30 to 11 A. M.		6.11[b]

* When the asterisk appears it signifies that the shoot was exposed directly to sunlight.

[b] When the letter *b* follows, it means that a bell glass was used over the shoot; otherwise the experiment was carried out in sunlight the same as in darkness.

reduced in 69 per cent. of the cases and in the willow in 59 per cent. A correlation therefore appears to obtain between the lighting conditions of the shoot and the rate of absorption of oxygen from the solution by the roots.

That there may be a shoot-root relation as regards oxygen absorption by roots is suggested by other observations. For example, when the possible effects of temperature and the evaporating power of the air were taken into account, no slowing of the rate was observed when the shoot was in darkness, or in a well lighted room, or in the shade of a tree, or on days with weak sunlight, or in the early forenoon, or in the late afternoon.

It appears, therefore, that in addition to proper conditions of illumination at least two other factors must be favorable in order to bring about the retardation of the rate of oxygen absorption. Inasmuch as the consumption of oxygen by the roots is a feature of respiration, it would be expected that its rate would depend in part, at least, on temperature. An increase in temperature would operate in the direction of increasing the rate of respiration, and a temperature decrease would have the opposite effect. That is to say, in the present instance the former temperature condition would induce a relatively rapid rate of absorption of oxygen by the roots while the latter condition would have a retarding effect. And such effects were noted. In addition, the temperature must be specifically favorable for the most active rate of respiration, which probably means that the temperature optima for the two species here used would not be the same, that of the willow being somewhat lower. Such conditions were recognized as being of importance in connection with the study, and as accounting for the effects of temperature, whichever way operating. Yet it was found that when temperature was favorable, and when the humidity of the air also was favorable, there was usually the slowing effect above noted, provided the shoot was properly illuminated.

It will be well here to speak of the effects of rapid evaporation on the absorption of dissolved oxygen by roots. Data from one or two experiments will illustrate this. Thus in one experiment with willow, the shoot was exposed to direct sunlight from 9 A. M. to 3 P. M. From 11 A. M. to 1 P. M. a current of air from a small electric fan was caused to play on the shoot. During the period when the fan was in use the rate of oxygen absorption increased about 50 per cent. In another and similar experiment the increase was about 200 per cent. In general it was found that when the rate of evaporation was fairly high, as often is the case in summer in this vicinity, the rate of oxygen absorption by the roots was relatively rapid. For this reason when the experiments were carried on in sunlight, negative results were often obtained; that is, there was no decrease in the rate of the consumption of oxygen by the roots. In many experiments with the willow, however, especially those in early spring, there was found to be a decrease in the rate of consumption of oxygen with the shoot in sunlight, notwithstanding the moderate evaportion rates then obtaining. But in no case, when the shoot was wilted, was there a slowing of the rate of oxygen absorption. The relation between the dryness of the air and the high rate of absorption of oxygen by roots was so constant that it was usually found necessary to protect the shoot with a bell glass. It can be said, therefore, that whenever the dryness of the air, or the temperature of the air or of the solution did not prevent, there was a distinct slowing of the rate of the removal of oxygen from the solution by the roots during the period in which the shoot was exposed to the sunlight.

In addition to the foregoing, the work of CERIGHELLI[5] might be cited as supporting the main thesis here advanced, although it is but fair to say that the interpretation of the results of that author are my own.

CERIGHELLI studied the respiration of the roots of different species of plants under different conditions. The following table, compiled from his results, summarizes the carbon dioxide-oxygen ratios, $\frac{CO_2}{O_2}$, expressed decimally, with the shoot in darkness and in light, as well as in dry and moist air.

PLANT	DARKNESS	LIGHT
Zea mays	0.68	1.11
Lupinus albus	0.60	0.80
Ricinus communis	0.88	1.07
" "	0.91	1.00
	Shoot in dry air	Shoot in moist air
Malva sylvestrum	0.19	0.62
" "	0.20	0.66
Phaseolus multiflorus	0.37	0.56

It appears, therefore, that CERIGHELLI found that there was relatively more oxygen consumed by the roots when the shoot was in the dark and also when it was in dry air, as opposed to the relatively small consumption when the shoot was in the light and also when in moist air. He accounts for the apparently, and perhaps actually, larger evolution of carbon dioxide, when the shoot is in light, on the assumption that "le gaz carbonique circulerait de la feuille a l' organe souterrain," as indicated by the larger ratio in light. But he gives no explanation of the analogous condition when the shoot is in moist air.

The interest centers in the variation in the carbon dioxide-oxygen ratios under the conditions of darkness and of light. Variation in intensity does not affect this ratio. As regards the difference in the ratios under the dark or light conditions, it is evident that two quite unlike processes may be dealt with. In the first case the only available oxygen to the roots, and probably also to the shoot, is the atmospheric oxygen either of the soil or dissolved in the solution containing the roots, or that of the atmosphere itself. But in the second case there is, in addition, the oxygen which may originate in the green tissues through photosynthesis. Should this oxygen, or a portion of it, remain within the plant and move away from the chlorophyll-bearing organs, it would play a part in the aeration of the plant, whether stem or root. In this event an apparent or an actual decrease in the consumption of oxygen by roots might be expected to take place. And, in fact, this would be indicated by an upset in the carbon dioxide-oxygen ratio by which the proportion of carbon dioxide given off exceeds that of oxygen taken in, as CERIGHELLI found. The ratio would thus be a large one owing not to a necessarily larger evolution of carbon dioxide but to a relatively small absorption of oxygen from the root substratum, for the reason that a portion of the oxygen used in respiration, during the periods of light, originated, as above suggested, within the plant itself. Thus to this extent the plant is autoaerated. In the present study a decrease in the rate of oxygen consumption under such conditions was determined, but in that of CERIGHELLI a larger ratio was found. Thus the two investigations apparently supplement each other and point to the same conclusion.

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[5] CERIGHELLI, R. Recherches physiologiques sur la respiration de la racine. Annales de la Faculté des Sciences de Marseille. 1921.

The finding of CERIGHELLI also that in dry air the ratio is smaller than when the shoot is in moist air harmonizes with the results of the present study, as will be concluded from data presented earlier. That is to say, it was found that the rate of absorption of oxygen by roots, in appropriate light, and with favorable temperature conditions was, however, greatly increased with increase in the rate of evaporation. Attention should be called here to the following facts. It is known that the rate of photosynthesis is more rapid in turgid than in flaccid leaves. Also it was found, when the water content of the leaves of *Bidens tripartita* was reduced to about 44 per cent., that the rate of photosynthesis was reduced from 53 to 78 per cent., and, in *Phlomis pungens* a reduction of 34 per cent. in the water content brought about a 13 per cent. reduction in the rate of photosynthesis.[6] From the present point of view the most interesting feature of these relations is the decrease in the rate of formation of oxygen which would accompany a reduced rate of photosynthesis, and thus a possible diminution in the amount of oxygen directly available to the plant, including the roots. The possible chain of events leading to this would include, among other features, (1) an excessive demand by the leaves on the roots for water leading to an inadequate supply; (2) a diminution in the water content of the parenchyma including the chlorenchyma; (3) the closing of the stomata; and (4) a diminution in or a cessation of the intake of carbon dioxide. There would be a decrease or a cessation of photosynthesis, and with it a diminution or complete stoppage of the formation of oxygen as a photosynthate. The closing of the stomata would also directly affect transpiration and possibly all molar movements within the

[6] MILLER, E. C. Plant Physiology. p. 467. 1931. See also SPOEHR, H. A. Photosynthesis. p. 153. 1926.

plant; and therefore the diffusion of oxygen from the chlorophyll-bearing organs to the stem and to the roots would cease, or occur at a slow rate. Thus there would be less oxygen available to the roots and there might be, as appears to occur, a correspondingly large demand on the substratum to make up the deficiency. This would not only bring about a relatively large loss of oxygen from the substratum (or, in this case, the solution), but it might, as CERIGHELLI showed, result in a relatively small carbon dioxide-oxygen ratio.

It thus appears probable from the results of the present study, as well as from those of CERIGHELLI, that a green plant may derive a portion of the oxygen used by it in oxidative processes from the shoot when the latter is in light of appropriate intensity, when the temperature is suitable, and when the evaporation conditions affecting the shoot are not intense. It is probable, therefore, that the root as well as the shoot is to a certain degree autoaerated. Oxygen of such derivation may for convenience be called "internal" oxygen, as opposed to atmospheric oxygen which enters the plant, including the root, without organic transformation.

The evidence given in earlier paragraphs that this internal oxygen may supplement atmospheric oxygen in the aeration of the roots, and of the stem, is, of course, inferential. There appears little experimentation bearing directly on the subject. Of these the most familiar is that with *Elodea* in which the cut end of the stem gives off oxygen when the shoot is in sunlight. But it was also shown in earlier studies by the writer that oxygen may pass from the shoot to the root under appropriate environmental conditions, especially with good light.[7] To secure more positive evidence a few experiments along the same lines were carried out in connection with this study. Pending further work, the leading results to this time may be briefly presented. Sunflower plants and cut leafy branches of apricot were tested with the root, or the cut end of the stem, in oxygen-free distilled water. The experiments were carried on in general quite as those already reported. It was found in several experiments that there was an increase in the oxygen content of the cultures coincident with the exposure of the shoot to sunlight.

Although the experiments just summarized are, as suggested, of an introductory nature, they appear to indicate (1) that the partial pressure of oxygen of the stem, and probably also of the root, may increase when the plant is in sunlight; and (2) that at the same time this oxygen may escape into the substratum surrounding the root. It was shown earlier in this paper also that the rate of the consumption of oxygen by the root, for parallel time periods, might be less when the shoot is in sunlight than when it is in darkness.

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[7] CANNON, W. A. Year Book, Carnegie Institution of Washington. no. 24. p. 292. 1925.

It has already been stated that positive results were not always had from the exposure of the shoot to sunlight. Thus not only must temperature and humidity be taken into account but certain features of light as well. Thus the length of exposure to light may be too short, or it may be too early in the morning or too late in the afternoon, or it may precede a period of darkness of equal length, or it may be too long. In the last case the period of most favorable rate of photosynthesis has been passed and in the next preceding case there may be photosynthetic oxygen in the tissues in sufficient quantity to influence the readings for the dark period. But in the instances first postulated the reasons for negative results are obvious. Although not investigated from the present point of view, it is not unlikely that different species may show unlike reactions to the light variations above mentioned.[8] However this may be, it appears that the processes associated with photosynthesis start promptly upon exposure of the shoot to light.[9] Where this feature makes itself evident in the stem, as indicated by the early appearance of oxygen in the cut branches of apricot, or by the early escape of oxygen from the root of the intact sunflower, it connotes a fairly rapid movement of oxygen from the chlorophyll-bearing tissue to relatively distant organs. This presents no difficulty, however, as movement of water and of solutes in the direction of, or against, the transpiration stream, and laterally as well, is known to occur,[10] although the details of the picture at least as regards oxygen translocation remain to be filled in.

Such considerations suggest the desirability of studies on the aeration of plant tissues. Oxygen so used appears to have a multiple origin. It may enter the plant as a gas directly from atmospheric air, or it may enter the roots dissolved in soil water, or it may arise as a product of photosynthesis. Thus oxygen from the substratum may conceivably aerate the root and perhaps the stem, and that from atmospheric air may aerate the stem and perhaps the root, and photosynthetic oxygen may perhaps aerate both stem and root. What the relative amount of oxygen taking its origin from the several sources, or in the different ways, may be, is quite problematical. It would surely vary with conditions and it may vary with the species. Certainly different species may not have the same light requirements, or the same oxygen requirements, and the possibility is that the two may be more closely related than is now commonly supposed. And, finally, it may well be that such specific differences in relation to oxygen in aeration may be important links in the chain of factors and events by which many species are adjusted to their special environment.

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[8] GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920. Also ADAMS, J. Relation of flax to varying amounts of light. Bot Gaz. **70**: 153–156. 1920, and later studies.

[9] *Cf.* CLEMENTS, H. F. Hourly variations in carbohydrate content of leaves and petioles. Bot. Gaz. **89**: 241–272. 1930.

[10] *Cf.* PEIRCE, G. J. The physiology of plants. p. 133. 1926, and MILLER, E. C. Plant Physiology. p. 669. 1931, for statements of the current views on movement of materials in plants.

STANFORD UNIVERSITY.

COMPARISON OF EXISTING METHODS FOR THE DETERMINATION OF AMMONIA NITROGEN AND THEIR ADAPTABILITY TO PLANT JUICE[1]

FRANK S. SCHLENKER

(WITH ONE FIGURE)

Introduction

In 1927 GILBERT and HARDIN (**4**), and later GILBERT and SMITH (**5**), published data showing that there is a correlation between soil fertility and the chemical composition of expressed plant juice. McCOOL and WELDON (**7**) and PETTINGER (**8**) in more recent work have substantiated the findings of GILBERT *et al.* The juice work of the Rhode Island workers has recently been extended to include the estimation of a number of the nitrogenous fractions. This has necessitated a study of methods, since the present chemical procedures used in plant work have been developed for water or alcoholic extracts of plant tissue. Accordingly the method for the determination of ammonia was chosen for adaptation to expressed juices, for the estimation of this plant constituent is one of the most frequent determinations made in the study of plants at this station.

Analytical methods

The initial ammonia methods used were those of FOLIN and BELL (**2**), the procedure as recommended by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (**1**), and that of VICKERY and PUCHER (**10**).

The first method involves the shaking of the sample with permutit which takes up the ammonium ion by means of a base-exchange reaction, and the subsequent liberation by sodium hydroxide; the second, aeration after making strongly alkaline with 52 per cent. potassium carbonate; and the third, an 8-minute distillation with heat after the addition of heavy magnesium oxide. In the final portion of the investigation, VAN SLYKE'S (**9**) aeration-distillation method, using a saturated calcium hydroxide solution for the neutralizing agent, was employed. With the exception of FOLIN and BELL'S technique which requires Nesslerization, the ammonia of the remaining procedures can be estimated either by the use of titration methods or Nessler's solution. The procedure of the VICKERY and PUCHER method was slightly modified. The use of permutit to remove ammonia from the distillate was discarded since no interfering substance, as nicotine

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in the original procedure, distills with the ammonia from plant juices. In addition the distillation was carried on by the use of an electric hot plate. By using an electric plate the amount of heat can be regulated more easily than with a micro-burner.

MATERIALS.—The plants for this study were obtained from the plats of the agronomy farm and from the college greenhouse. The leaves were treated as recommended by FREAR (3), except that after being wrapped as tightly as possible in cheesecloth they were placed in the carbo-freezer and frozen by a direct stream of carbon dioxide rather than indirectly by dry-ice cakes. After complete thawing the juice was expressed in a hydraulic press.

Experimental

In order to obtain an idea of the composition of the material to be used, a few miscellaneous determinations were made; that is, the weight of 5 ml. of juice, the hydrogen-ion concentration, ash, the approximate ammonia content of mesophyll and midrib tissue were secured (table I). Mesophyll tissue, as discussed here, is that portion of the leaf remaining after removal of the midrib. This includes, besides mesophyll tissue proper, the lateral veins which are too small to remove.

TABLE I

DETERMINATION OF SOME JUICE CONSTITUENTS (BEETS)

	WEIGHT OF 5 ML. OF JUICE		ASH		AMMONIA NITROGEN		HYDROGEN-ION CONCENTRATION	
SAMPLE NO.	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB	MESOPHYLL	MIDRIB
	gm.	*gm.*	*per cent.*	*per cent.*	*p.p.m.*	*p.p.m.*	*pH*	*pH*
1	5.0873	5.0980	. .		24.4	24.0	6.50	5.71
2	5.0779	5.0799	2.05	1.89	14.1	13.7	6.42	5.79
3	5.1005	5.1003	1.88	1.87	20.1	19.9	6.33	5.71
Average	5.0885	5.0927	1.96	1.88	19.5	19.2	. .	.

It has been the usual practice to use a 5-ml. sample of juice and consider the weight as 5 grams, for the sampling error is considered to be greater than the error involved by assuming this weight. Actually in a juice containing 50 p.p.m. of ammonia nitrogen the error is approximately 1 p.p.m., or 2.0 per cent.

The procedure as recommended by the Committee on Methods (1), which for convenience will be termed the aeration method, has been used for the ammonia determinations from the beginning of the fractionation of nitrogen in plant juice at this station.

Kober and Graves (6) cite evidence which shows that the amount of ammonia carried over by a stream of air depends on the volume and rate of aeration. They found that 650 liters of air per hour recovered 99.7 per cent. of ammonia from an ammonium sulphate solution in 50 minutes, while 139 liters per hour recovered 97.4 per cent. in 90 minutes. To cover this portion of the procedure, a flow meter was calibrated by a Junkers gas meter and a curve for the relationship of time and volume constructed. The flow meter was then inserted in the system, the suction adjusted to 110–120 liters per hour, and the aeration continued for two hours.

The 2-hour aeration period is considerably more than that mentioned in the original method, but experimental results indicate that this length of time is necessary to carry over the ammonia present at this rate of aeration. In an effort to follow the course of the aeration reaction, time-concentration studies were made for the juices used in this study and for a solution of ammonium sulphate. The ammonia in an ammonium sulphate solution can be recovered quantitatively in a short period of time. The results for plant juices when plotted (fig. 1) show that at the end of two hours there is a distinct change in the type and rate of reaction, for at this point there is a break in the curve and the evolution of ammonia becomes less rapid thereafter. The curve for beet juice is the most striking, bringing out more clearly than the other two curves the important point to be derived from these experiments. The ammonia results obtained by aerating for two hours showed close agreement with those obtained by the use of permutit as discussed in the succeeding paragraph. As the change in the rate of evolution of ammonia appears at that point where the results of the two determinations coincide, it can be concluded that two hours' aeration is sufficient to measure the uncombined ammonia.

With the idea in mind that the continued appearance of ammonia with prolonged aeration might be due to the hydrolysis of some unstable nitrogenous compounds, permutit was employed to remove the ammonia but not the unstable nitrogenous products. Folin and Bell (2) demonstrated that ammonia could be removed quantitatively from urine by the use of permutit, even in the presence of 20 to 30 mg. of sodium chloride. Whitehorn (11) found that substances other than ammonia were taken up by permutit. The most important of these were the amines and amino acids, lysine, histidine, and portions of tyrosine and arginine. As Folin pointed out that the extraction of ammonia from solution was limited by the concentration of sodium chloride, it was necessary to test the adsorbing power of permutit in a solution having an inorganic salt concentration approximating that of plant juice. It will be seen from table I that the ash content of plant juice is between 1.96 and 1.88 per cent., and this gives an average concentration of 19 mg. of inorganic salts per ml. of juice, a figure close to **Folin**'s lower limit. The ash content does not measure the salt concentration exactly, because of the evolution of some of the more volatile inorganic constituents of the juice, so that these figures are probably low.

Two buffered salt solutions were made up to have hydrogen-ion and salt concentrations somewhat similar to the plant juices in question. In table II are recorded the results for recovery of ammonia by permutit adsorption.

TABLE II

RECOVERY OF AMMONIA NITROGEN BY PERMUTIT FROM SYNTHETIC SOLUTIONS CONTAINING 50 P.P.M. OF AMMONIA NITROGEN

	SOLUTION 1,* pH 6.20	SOLUTION 2, pH 5.49
	per cent.	*per cent.*
	97.50	99.60
	101.00	98.70
	99.50	100.00
	100.60	99.20
	99.50	100.00
	100.00	99.20
	98.50	100.00
	100.00	99.00
	99.50	99.50
Average	99.56	99.46

Solution A: sodium monohydrogen phosphate 11.857 gm. per liter.
Solution B: potassium dihydrogen phosphate, 9.078 gm. per liter.

Synthetic solution 1: pH 6.20, 50 p.p.m. ammonia nitrogen

Sodium monohydrogen phosphate (A) 150 ml.	1.770	gm.	
Potassium dihydrogen phosphate (B) 350 ml.	3.185	''	
Ammonium sulphate	0.097	''	
Sodium chloride	5.000	''	
Total inorganic salt concentration, 2.01%	10.052	''	in 500 ml.

Synthetic solution 2: pH 5.49, 50 p.p.m. ammonia nitrogen

Sodium monohydrogen phosphate (A) 37.5 ml.	0.443	gm.	
Potassium dihydrogen phosphate (B) 462.5 ml.	4.209	''	
Ammonium sulphate	0.097	''	
Sodium chloride	5.000	''	
Total inorganic salt concentration, 1.95%	9.749	''	in 500 ml.

In so far as the two mixtures can be considered representative of plant juice with respect to hydrogen-ion and salt concentration, there is no doubt that permutit can be used to remove ammonia from solution quantitatively.

FOLIN's method as adapted to juice work consisted of shaking 5 ml. of juice with 2 gm. of washed permutit in a 50-ml. volumetric flask for 5 to 7 minutes, washing free from remaining juice by decantation, adding 20 ml. of water and 2 ml. of 10 per cent. sodium hydroxide, then 5 to 10 ml. Nessler's reagent and diluting to volume. In all cases where this method was used an "off" color was produced which gave varying intensities of yellow light. This interfering color seemed to vary with crops, being less noticeable for beets and more for celery. In some instances, with celery, after the release of ammonia by sodium hydroxide the supernatant liquid showed a decided yellow color which disappeared upon addition of acid. It is not soluble, however, in such organic solvents as caprylic alcohol, ether, or xylene. In spite of this interfering substance the method can be used with a fair degree of accuracy, provided the "off" color is not too great, after becoming accustomed to the point of color match. This particular point seems to be that place where there is a sudden change from light yellow to a darker shade of the same color in the unknown solution, when comparing it with the standard solution in a Duboscq colorimeter. Before this procedure can be adopted with a perfect degree of surety this difficulty must be overcome, but the color interference does not entirely dismiss the use of permutit. It may be useful as a rapid method if great accuracy is not required.

The permutit method may be made accurate by the addition of aeration to the procedure as follows: 2 gm. of washed permutit are placed in an aeration tube and 5 ml. of juice added. This is shaken for 5 to 7 minutes and then washed clear of remaining juice by decantation. The tubes are placed in the VAN SLYKE-CULLEN aspiration block, and 3 ml. of 10 per cent. sodium hydroxide and an anti-foam reagent added. The apparatus is then connected as usual and aspirated 90 minutes (table III). This is the minimum time that can be used with safety. The ammonia is caught by 3 ml. of 0.01 N acid and either transferred to a volumetric flask for Nesslerization and dilution, or titrated by standard base.

With any of the methods where distillation or aeration are involved anti-foam reagents must be used. Ninety-five per cent. alcohol in the VAN SLYKE and paraffin in the VICKERY and PUCHER method works very well. In the aeration method caprylic alcohol is efficient, but it distills into the acid tube readily, and upon the development of color by Nesslerization a cloudiness appears, provided too much alcohol is present. If, however, the color is not brought out until just before comparison, with quick manipulation the colors can be matched successfully. This latter procedure was

TABLE III

TIME NECESSARY TO LIBERATE AMMONIA FROM PERMUTIT BY ASPIRATION

MINUTES	JUICE AMMONIUM NITROGEN			PURE AMMONIUM SULPHATE SOLUTION 50 P.P.M. AMMONIUM NITROGEN	
	BEETS	BEETS	CELERY		
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
10	6.70	7.00			
20	10.00	12.10		26.60	
30	12.50	14.70		38.50	
60	14.50	25.00	156.0	41.60	39.60
70			178.5		41.70
80			178.5	50.00	49.00
90			178.5		50.00
100					50.00
120	14.50	25.00			

found to be inconvenient so other organic compounds were tried. The most useful one was a mixture of paraffin dissolved in xylene. This combination has no effect on a Nesslerized solution.

Although recovery experiments are not absolute proofs of the accuracy of a method, they are helpful. Table IV shows that all of the methods tested recovered practically all of the ammonia added.

COMPARISON OF METHODS

After becoming familiar with the various methods, they were used as nearly simultaneously as possible on juices from different plant species. The results obtained by the methods are given in table V. For convenience in comparison FOLIN's method was arbitrarily chosen as the standard. The relationships are determined in the following manner. The individual results in any one column and the corresponding results by FOLIN's permutit method are averaged separately and the relationship between the two is calculated, using the average obtained by the FOLIN method as a base of 100.

An examination of table V indicates the relative values to be expected when any of the methods are used. The FOLIN and aspiration methods give closely comparable results; the modified permutit and VAN SLYKE methods tend toward lower results which are also comparable; while those obtained by VICKERY and PUCHER's method are high. The differences between the methods are probably within the errors of sampling, so that any one of the tested procedures can be used. It appears, however, that VICKERY and PUCHER's technique as applied to expressed plant juice probably approaches the limits within which these procedures are applicable.

TABLE IV

PERCENTAGE OF RECOVERY OF AMMONIA NITROGEN ADDED TO PLANT JUICE BY VARIOUS METHODS

	FOLIN PERMUTIT		AERATION				VICKERY AND PUCHER				VAN SLYKE	
	CELERY	BEETS	CELERY		BEETS		CELERY		BEETS		CELERY	BEETS
	NESSLER	NESSLER	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	NESSLER
	103.0	95.0	101.0	102.0	92.6	96.5	105.0	99.3	99.7	...	101.0	100.0
	98.5	94.3	103.0	98.5	97.2	96.4	98.4	96.8	97.0	...	101.0	97.9
	103.0	96.0	103.0	107.0	96.9	...	99.0	93.5	99.0	...	95.0	97.9
	101.0	...	100.0	97.0	103.7	...	98.5	101.5	100.5	...	98.0	...
	...	...	97.5	99.0	103.0	...	98.5	.	99.5	.	...	...
	...	...	99.0		103.2	...	..	.	100.0	...	...	...
	.	...	101.0	...	...	..		..	.	...	...	...
Average	101.3	95.1	100.6	100.7	99.5	96.4	99.9	98.0	99.2	..	98.7	98.6

TABLE V

COMPARISON OF AMMONIA NITROGEN IN PLANT JUICE AS DETERMINED BY VARIOUS METHODS

		FOLIN	MODIFIED PERMUTIT		ASPIRATION		VICKERY AND PUCHER		VAN SLYKE
		NESSLER	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER	TITRATION	NESSLER
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Beets	1	26.7				24.9	26.7	27.4	
	2	6.8					6.9	9.5	
	3	6.0					6.1	10.9	
	4	6.5					6.5		
	5	19.5				24.1	20.0	23.5	
	6	10.3				9.8	10.5	13.7	
Celery	7	10.0			13.3	14.0	17.0	14.8	
	8	13.8			16.7	17.1	19.0	19.6	
	9	13.3			13.2	14.8	15.1	20.0	
	10	16.7			17.0	15.4	22.0	26.0	
	11	11.1			15.8	15.1	24.0	20.5	
	12	12.5			12.5	9.0	15.7	13.4	
	13	11.5			11.5	8.1	19.6	16.0	
Beets	14	14.9	14.3	14.3	15.9	14.3	19.8	18.5	15.0
	15	33.4	31.2		33.4		35.3		32.9
	16	80.0	75.8	74.8	84.8	79.1	86.2	86.5	73.5
Celery	17	40.3	37.8	35.3	40.2	44.8	45.5	46.8	24.5
	18	80.6*	57.1		55.5		62.5		55.6
Spinach	19	50.0	47.0	45.7	47.0	46.6	51.2	53.0	47.5
	20	80.0	78.3		78.3		83.5		74.8
	21	57.6	53.0		52.5		62.5		54.3
	22	44.2	37.8	31.4	39.3	39.5	48.5	50.0	41.0
Average p.p.m.		50.0	46.9		48.3		53.9		46.7
Percentage of FOLIN method		100.0	94.0	88.2	100.4	100.4	110.6	121.0	90.7

* This result is much too high, owing to the amount of interfering yellow pigment. In all cases it and the corresponding values in the other columns have been left out of the calculations of relationship of the other methods to the FOLIN method.

Discussion

The results in table V are shown relatively, with no concern as to their accuracy for plant use. Of all the methods described, it is the writer's belief that Folin's permutit method, as modified in this investigation, is the most accurate. If any of the usual distillation or aeration methods are allowed to proceed, the evolution of ammonia continues indefinitely. This makes it necessary to add a time factor to these procedures to avoid the inclusion of ammonia due to the hydrolysis of metabolized nitrogen. This difficulty is overcome by the use of permutit followed by aeration. The permutit removes an amount of ammonia which corresponds to that given off up to the time when there is a break in the time-concentration curve (fig. 1), and subsequent aeration removes the ammonia free from any substance which interferes with Nesslerization. There is, however, a possible source of error in this method. As stated before, Folin has shown that in the presence of a certain salt concentration permutit does not function perfectly, and it is possible that a juice may have this inorganic salt concentration, thus inhibiting its use. The inorganic salt concentrations of the juices used in this study lend themselves to the use of permutit. For expressed juices which contain prohibitive amounts of inorganic salts, dilution may be used to overcome this factor.

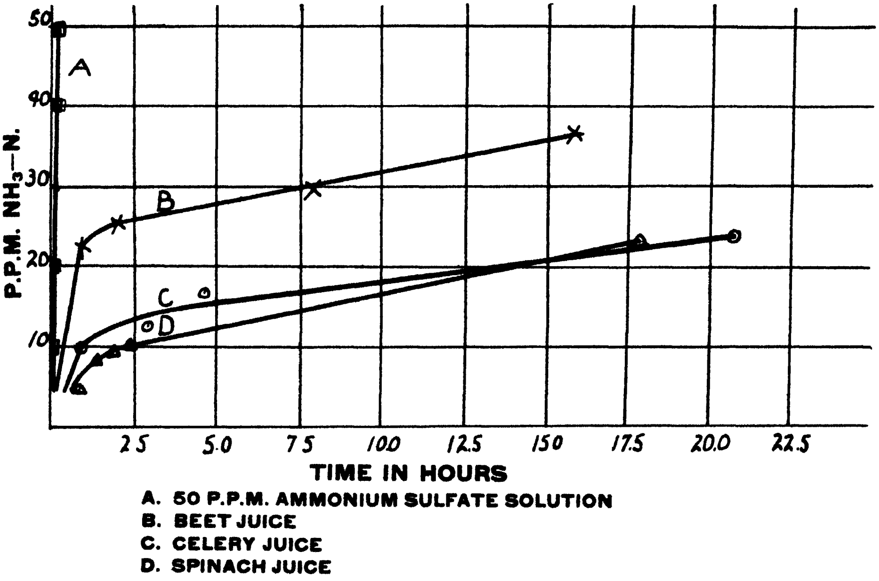

Fig. 1. Graph representing amount of ammonia nitrogen, in parts per million, aerated with time.

Summary and conclusions

1. Five methods for the determination of ammonia have been compared, all of which give rather close agreement.

2. Folin's permutit method readily adsorbed not only ammonia from plant juice but also a yellow pigment. This pigment, like ammonia, was released from permutit upon the addition of sodium hydroxide.

3. A new procedure combining the use of permutit and aeration gives reliable results and overcomes the difficulties of adsorbed pigments and hydrolyzable substances.

4. All methods recovered known amounts of added ammonia quantitatively.

The writer wishes to express his appreciation to Mr. John B. Smith for his assistance during the course of this investigation.

Rhode Island Agricultural Experiment Station,
Kingston, R. I.

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MANGANESE ESSENTIAL FOR THE GROWTH OF *LEMNA MAJOR*[1]

J. S. McHargue and R. K. Calfee

(WITH THREE FIGURES)

Previous reports (**3–8**) from this laboratory have shown that manganese is an essential element for the normal growth and maturation of important agricultural crops, and that it has an important function in the synthesis of chlorophyll. Our experiments were carried on in both water and purified sand cultures. In our former reports attention was called to the more common sources of manganese contamination and the necessity of eliminating traces of this element from plant nutrients, distilled water, and vessels in which plants are to be grown.

In our previous work, experiments to show the necessity of manganese for plant growth were made with land plants which have some economic importance and a much more complex morphological structure than *Lemna major.*

Lemna major (*Spirodela polyrhiza*) is a minute, stemless, free-floating, aquatic plant consisting of a mere frond from which, on the under side, grow roots about 10 mm. long. The plants propagate by the proliferous growth of a new individual from a cleft in the edge or base of the parent frond, and also by autumnal fronds in the form of minute bulblets which sink to the bottom of the water during the winter season but rise to the top and vegetate in the spring.

Clark and Fly (**1**) have recently stated that manganese is not necessary for the growth of *Lemna major.* Because this plant synthesizes chlorophyll, we became doubtful of the findings of these investigators, and have carried on experiments for the past six months to ascertain to our satisfaction whether or not manganese is essential for the growth of this plant. Normal plants were collected from the surface of a pond about the middle of July, 1931, and transferred to water cultures contained in acid-proof stoneware jars. The solutions in which the plants were grown contained the following amounts of mineral plant nutrients:

Calcium nitrate, $Ca(NO_3)_2.5H_2O$	4.0 gm.
Potassium nitrate, KNO_3	1.0 ”
Magnesium sulphate, $MgSO_4.7H_2O$	1.0 ”

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[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Potassium acid phosphate, KH_2PO_4	1.0 "
Potassium chloride, KCl	0.5 "
Ferric citrate, $FeC_6H_5O_7.3H_2O$	0.1 "
Distilled water	7 liters

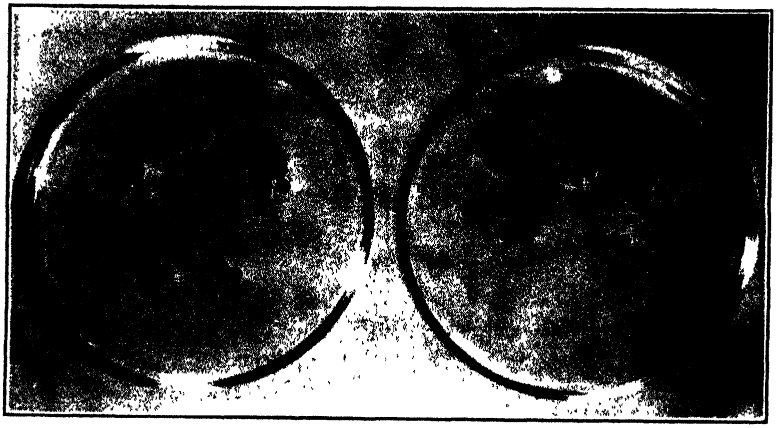

FIG. 1. Influence of manganese on the size and color of *Lemna major:* plants on left were grown in a solution containing 0.25 p.p.m. manganese; plants on right are manganese-deficient. 4/5 actual size.

The salts used in preparing this solution were tested chemically to ascertain their freedom from a number of the less common elements, manganese included. The water was distilled and condensed in a quartz tube, and collected and stored in an acid-proof stoneware receiver. To avoid possible complications from the absence of elements other than manganese, 0.5 part per million of boron, and 50 parts per billion each of copper and zinc were included in the manganese-free medium.

Plants from a stock culture of *Lemna* (containing manganese) were transferred to two jars of this medium, one of which had received enough manganese sulphate to bring the manganese content to 0.5 part per million but no manganese was added to the second jar. There was no discernible difference in the growth or appearance of the two cultures at the end of eight weeks. Plants in both cultures had practically covered the surface of the solutions. A few plants from each culture were then transferred to fresh solutions of corresponding composition. At the end of eight weeks more the appearance of the plants in the two cultures was practically identical, but the rate of growth was unequal, as evidenced by the somewhat crowded condition of the plants in the manganese-treated culture as compared with the more sparse growth of plants in the manganese-deficient culture. Transfers were again made to fresh solutions. Normal growth occurred in the manganese-treated culture, the plants covering all available space in eight weeks. The plants in the manganese-deficient culture appeared to be normal for two weeks, but during the third week many of the fronds were notably lighter in color and smaller in size. Definite chlorosis was present in this culture by the end of the third week. Figure 1 compares the size and gives some indication of the difference in color of representative plants of the treated and deficient cultures. At the beginning of the fifth week several of the fronds were dead, necrotic areas were present on many others, and all were chlorotic. The living plants at this time were separated into two equal groups, forming two cultures. Ferric nitrate (0.0005 gm. per liter, manganese-free) was added to the first culture, but failed to relieve the condition, and the plants died. Manganese sulphate at the rate of 10 parts per billion was added to the second culture of chlorotic plants. Many plants in this culture, those that showed extreme chlorosis, died. The majority, however, slowly regained some of their original green color and within eight days had resumed growth and production of new fronds. This culture was reserved as a stock culture for further experiments. The volume was kept up by the addition of a manganese-free nutrient solution and traces of manganese were added at the first evidence of chlorosis. The rate of reproduction in this culture was considerably below that of the cultures containing an abundance of manganese.

To ascertain the influence of manganese on the reproduction of *Lemna,* two groups of six jars were started with one frond each, selected for uniformity of size and color, from the culture of low manganese content. The total number of fronds was counted at the end of each week, for eight weeks. The average of each group is shown in figure 2.

Because of the small increase in weight that manganese-deficient cultures made before death, it was impracticable to produce sufficient material for a complete chemical analysis. By greatly increasing the size of the stock cultures and by using a large number of fronds to start the manganese-deficient cultures, enough material to determine ash, manganese, and iron eventually was obtained. Abundance of material was available without difficulty from the rapid-growing manganese-treated cultures. The results are given in table I, calculated as percentages of the moisture-free plants.

The failure of *Lemna* to make normal growth in a nutrient solution free from manganese apparently was due to a disturbance of metabolism affecting the chlorophyll, resulting in chlorosis, followed by necrosis and death of the plant. The rate of reproduction was affected pronouncedly by the manganese content of the culture. This effect was more marked after several weeks of growth, largely because of the greater number of plants capable of producing new fronds in the manganese-treated cultures, and also because of the slightly higher rate of growth of the individual fronds. At the end

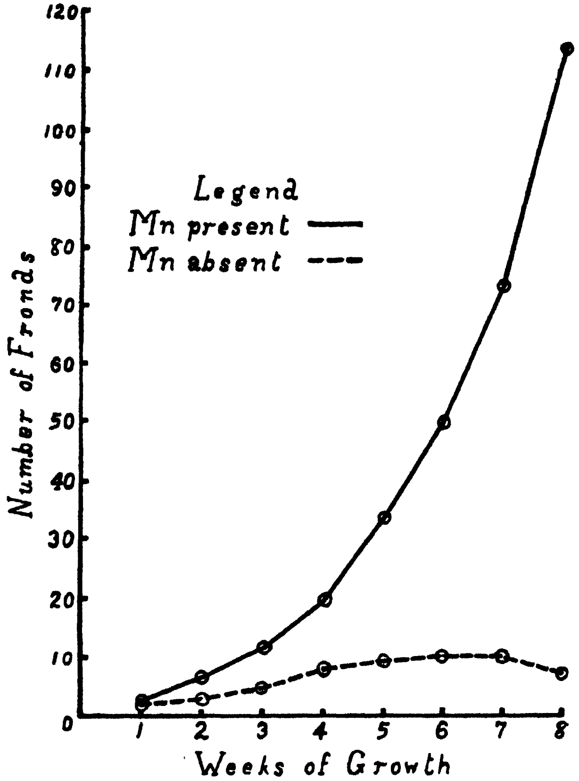

FIG. 2. Effect of manganese on rate of growth and reproduction of *Lemna major*.

TABLE I

ASH, MANGANESE, AND IRON CONTACT OF *Lemna* GROWN IN DIFFERENT MANGANESE CONCENTRATIONS

	MN, ABSENT (DEFICIENT)	MN, 7 P.P.B. (MAINTENANCE)	MN, 250 P.P.B. (OPTIMUM)
	per cent.	*per cent.*	*per cent.*
Ash	18.575	19.342	21.080
Mn	0.005	0.009	0.059
Fe	0.172	0.155	0.070

of the first week, the manganese-deficient cultures contained practically the same number of fronds as the manganese-treated cultures. At the end of the fourth week, the plants in the manganese-deficient cultures were chlorotic and reproduction had practically ceased. By the end of the sixth week some of the fronds were dying. The nature of the plant made it impossible to determine whether this difference in the rate of reproduction was due directly to the absence of manganese or to the starved condition of the plant which resulted from manganese deficiency.

The total mineral content of the fronds was decreased by the absence of manganese in the culture. The manganese content of chlorotic and necrotic plants was less than one-tenth that of normal plants. The amount of manganese was small but was sufficient to be determined in a colorimeter. The iron content of the plants increased as the manganese decreased, suggesting an attempt at substitution on the part of the plant.

Investigations in this laboratory with *Lemna major* proved it to be rather unsatisfactory material for experimentation. Since it is a floating plant, the surface of the medium must be greater than the leaf surface produced, and as the fronds constitute practically the entire weight of the plant, the dry material obtained is much less than most experimental plants would produce on equivalent bench space. The plant consists essentially of roots and leaves, so evidence of deficiencies or toxicities must be obtained from the effects on size and color of the fronds, condition of the roots, and the rate of reproduction. Figure 3 shows the relative size and the parts of the plant. New fronds are produced from older fronds from buds, or special cells. This method of reproduction is not favorable for investigation of deficiencies that may influence the rate of reproduction.

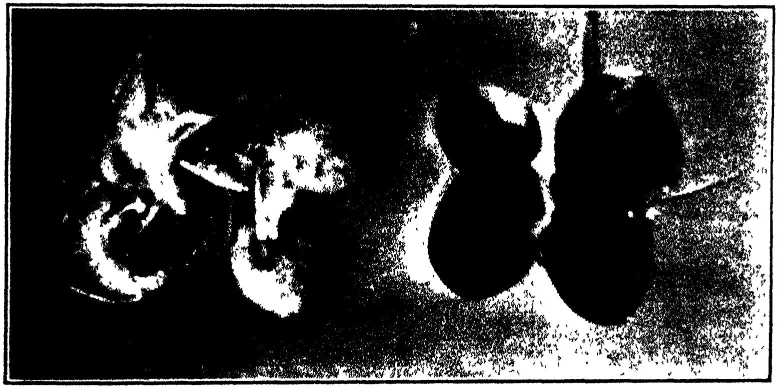

FIG. 3. *Lemna major:* left, under side showing roots and manner of producing new fronds; right, top view. Magnified about 4 times.

Since our experiments with *Lemna major* were begun, HOPKINS (2) has published results showing that manganese is necessary for the growth of *L. minor*. Our results on *L. major,* although obtained by a somewhat different procedure, confirm his findings.

Clark and Fly, in referring to previous work by the senior author (8), misquote him in a way that requires correction. The statement of Clark and Fly is as follows: "He [McHargue] suggests here that it is the manganese which causes the deep blue color of blue grass, etc." The writer in the article referred to makes this statement, however: "Kentucky bluegrass is particularly rich in manganese and chlorophyll and therefore affords an interesting example of the function of manganese in the development of the beautiful deep *green* color which is characteristic of the luxuriant bluegrass pastures." Doubtless many persons who have visited the bluegrass region of central Kentucky have wondered where the blue is in *Poa pratensis*. Incidentally, we commend the wisdom of the Committee on Standardized Plant Names, in making one word of bluegrass.

Summary

1. Fronds of *Lemna major* were grown in nutrient solution with and without manganese.
2. A deficiency of manganese in the nutrient solution resulted in retarded growth, failure to reproduce, chlorosis, necrosis, and death after utilization of the residual metal in the plants.
3. Chlorotic plants in manganese-deficient solutions were restored to their original dark green color by the addition of traces of manganese sulphate but not by ferric nitrate.
4. The exclusion of manganese from the medium resulted in a decrease in the total ash content and an increase in the iron content of the plant.
5. Because of its inherent characteristics, *Lemna* was found to be less satisfactory than most experimental plants for greenhouse cultures.

Department of Chemistry,
Kentucky Agricultural Experiment Station,
Lexington, Ky.

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CARBOHYDRATE CONTENT OF TOMATO FRUIT

L. G. SAYWELL and D. P. ROBERTSON

The carbohydrate content of mature tomato fruit has received considerable attention since ATWATER and BRYANT (**1**) indicated the total sugar content to be about 3.39 per cent. and the starch less than 0.1 per cent. on the fresh basis. In a later publication of the same series containing revised data, CHATFIELD and ADAMS (**6**) gave the average reducing sugar content as 3.37 per cent. MYERS and CROLL (**11**) reported the reducing sugar content of two samples of tomatoes to be 3.39 and 2.91 per cent. and the total sugar content 3.47 and 3.25 per cent. BELL, LONG, and HILL (**2**) determined the available carbohydrate content of two samples to be 2.6 and 3.0 per cent., respectively. LAWRENCE and MCCANCE (**9**) have placed it at 2.4 per cent. ROSA (**13, 14**) has presented a study of the composition of tomatoes at different stages of maturity. For the mature fruit, the reducing sugar content of five samples varied from 2.63 to 3.80 per cent. For two of these the sucrose content, by invertase inversion, was 0.04 and 0.11 per cent. The starch content, by diastase inversion, varied from 0.012 to 0.52 per cent. for the five samples. For the same two samples upon which sucrose determinations were made the values for acid-hydrolyzable material, by hydrolysis with 5 per cent. hydrochloric acid, were 0.180 and 0.212 per cent. respectively. BENOY and WEBSTER (**3**) have reported that the total and reducing sugar concentrations were equal. They reported values of 2.43 per cent. for each, and 0.069 per cent. of starch on the fresh basis. BIGELOW and STEVENSON (**5**) and BIGELOW and FITZGERALD (**4**) have studied the chemical composition of commercial tomato products prepared in the eastern tomato-growing sections of the United States. Their values for reducing sugar content of filtrates from pulps averaged about 4.20 per cent. CRUESS, SAYWELL, and HARK (**8**) have given preliminary data on the composition of California tomatoes.

The present investigation was undertaken in order to secure a more complete knowledge of the carbohydrate composition of California tomatoes. The plan has been to study the composition of composite samples of both the entire fruit and the several more or less distinct botanical regions of the fruit. Determinations have been made of the reducing sugars, sucrose, starch, and acid-hydrolyzable material and the dextrose-levulose ratios.

Materials and methods

Large samples of from 15 to 20 previously frozen fruits were taken to reduce sampling error. Each fruit was quartered through the polar diameter and two alternate quarters were used for a composite sample. When it was desired to have samples of the core, locule, interlocular septa and outer walls, the remaining quarters were cut through the equatorial diameter and the required portions then carefully secured. The samples were then ground in a food chopper and analyzed at once or were placed in cold storage (0° C.) if the analysis could not be completed immediately.

Reducing sugars were determined by the SHAFFER-HARTMAN method (**15**) after clarifying with saturated neutral lead acetate and deleading with sodium oxalate. Dextrose was determined by the method of LOTHROP and HOLMES (**10**). Sucrose was inverted with invertase for one hour at 55° C. The total reducing sugars present then were determined and the sucrose content calculated by difference. Starch was determined by the following modification of OLMSTED's method (**12**). To a 25-cc. aliquot of the sample 0.1 gram of Taka-diastase was added. After careful mixing, the solution was brought to 55° C. and held at this temperature for one hour. After cooling and clarifying, the reducing sugars were determined as previously described. The acid-hydrolyzable carbohydrate material was determined by direct acid hydrolysis (**16**). This fraction includes the starch. Proper blanks were made for all determinations.

Total solids were calculated from the refractive index-total solids ratio determined by CRUESS and SAYWELL (**7**) for California tomatoes.

Results and discussion

For five samples analyzed, the sucrose content was 0.03, 0.05, 0.04, 0.02, and 0.07 per cent. respectively. Since these values were fairly uniform and all less than 0.10 per cent. it appeared that the sucrose content of mature fruit was relatively small and frequently less than 0.05 per cent. Consequently further determinations were not made. However, it is not intended to preclude the possibility of important physiological functions of sucrose.

Starch determinations were made on the above five samples. Values of 0.045, 0.038, 0.040, 0.031, and 0.037 per cent. starch were obtained. The first two samples were from vines of the San Jose Canner variety, the third and fourth from Santa Clara Canner vines, and the fifth from Stone. ROSA (**13, 14**) has reported values of 0.044, 0.052, 0.022, 0.034, and 0.012 per cent. on samples of ripe fruit of Earliana, Globe, and Stone varieties. Since the two series of results agreed quite closely, it appeared that the usual range of starch content of mature fruit was from 0.02 to 0.05 per cent., expressed on the fresh basis.

In order to secure data on reducing sugars representative of the state, samples from the more important tomato producing districts were analyzed with the results given in table I.

It would appear from table I that the average reducing sugar content of California tomatoes is somewhat higher than that generally recorded in the literature for other localities. Considerable variation was found in the

TABLE I

REDUCING SUGAR CONTENT OF MATURE TOMATO FRUIT GROWN IN CALIFORNIA (EXPRESSED AS THE PRECENTAGE OF THE FRESH WEIGHT)

LOCALITY	REDUCING SUGARS	TOTAL SOLIDS
Arlington	3.28	6.57
Buena Park	3.62	8.13
Burbank	3.45	7.30
Centerville	4.00	8.05
Chino	3.04	6.88
Davis	3.54	6.93
El Monte	3.38	6.42
Fullerton	3.57	7.41
Hayward	3.81	7.22
Locke	3.96	7.22
Riverside	2.95	6.05
Sacramento	4.01	6.82
Salinas	3.94	6.82
San Fernando	3.33	6.43
San Jose	3.66	6.92
Santa Clara	3.38	6.30
Sunnyvale	3.80	7.03
Average	3.57	6.97

total solids and reducing sugars of samples from different districts within the state.

The results of the analyses of the cores, the locules, the interlocular walls and outer wall portions of alternate quarters of the fruit and a corresponding composite of these regions prepared from the remaining quarters are given in table II. Samples were taken October 2, 1931.

From these data it is apparent that the tissue of the core region is relatively high in reducing sugars, total solids, and acid-hydrolyzable contents. The locular material is considerably lower in total solids, reducing sugars, and acid-hydrolyzable material than any other portion of the fruit. The acid-hydrolyzable content is very much lower in the locular material. The walls approach the core and composite in composition except that they are somewhat lower in reducing sugars.

The results of the dextrose-levulose and total solids determinations on representative samples from several of the important tomato producing regions of the state are given in table III. It is interesting to note that the dextrose-levulose ratios generally are quite uniform and that the average levulose content is about 46 per cent. of the average total reducing sugar content.

Summary

The sucrose content of California tomatoes varied from very small quantities up to 0.05 per cent. and the reducing sugar content, from 3.30 to 3.70 per cent.; the dextrose content was about 1.2 times the levulose content. The starch content varied from 0.01 to 0.06 per cent., while the acid-hydrolyzable material varied from 0.10 to 0.30 per cent. The total solids content ranged from 6.5 to 7.0 per cent. on the average. All results are expressed on the basis of the fresh weight of the fruit.

TABLE II

COMPOSITION OF DIFFERENT REGIONS OF TOMATO FRUIT (EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT)

SAMPLE NUMBER	REGION	REDUCING SUGARS	TOTAL SOLIDS	ACID-HYDROLYZABLE
		per cent.	*per cent.*	*per cent.*
1–1	Core	2.90	7.1	
	Locule	2.69	6.6	
	Walls	2.32	6.6	
	Composite	2.70	6.6	
1–2	Core	3.30	6.3	
	Locule	2.90	6.0	
	Walls	2.96	6.2	
	Composite	3.08	6.2	
2–1	Core	3.18	6.2	
	Locule	2.72	5.9	
	Walls	3.04	6.2	
	Composite	3.10	6.2	
2–2	Core	2.60	5.8	
	Locule	2.13	5.1	
	Walls	2.37	5.5	
	Composite	2.49	5.6	
1–3	Core	2.94	6.2	
	Locule	2.52	5.8	
	Walls	2.79	5.8	
	Composite	2.87	5.9	
1–4	Core	2.66	6.0	
	Locule	2.07	6.0	
	Walls	2.53	5.8	
	Composite	2.62	5.9	
1–5	Core	3.36	6.3	
	Locule	2.86	6.2	
	Walls	2.84	6.0	
	Composite	3.13	6.2	
2–2	Core	2.86	5.0	
	Locule	2.34	4.9	
	Walls	2.39	5.0	
	Composite	2.58	5.0	
2–4	Core	2.61	5.2	——
	Locule	2.13	5.0	——
	Walls	2.38	5.0	——
	Composite	2.48	5.0	——
3–2	Core	3.47	5.2	——
	Locule	3.15	5.1	——
	Walls	3.76	5.1	——
	Composite	3.67	5.2	——
1–1–2	Core	3.61	6.3	0.245
	Locule	3.26	6.2	0.055
	Walls	3.30	6.2	0.265
	Composite	3.49	6.2	0.210
1–2–2	Core	2.77	6.3	0.320
	Locule	2.30	5.8	0.060
	Walls	2.85	6.2	0.240
	Composite	2.96	6.2	0.235
Marglobe (1)	Core	3.05	7.1	0.260
	Locule	2.53	7.2	0.065
	Walls	2.73	7.2	0.282
	Composite	2.92	7.2	0.275
Santa Clara (3)	Core	3.30	5.1	0.210
	Locule	2.98	5.0	0.050
	Walls	3.07	5.0	0.370
	Composite	3.23	5.1	0.320
Santa Clara (2)	Composite	2.67	5.1	0.295
Marglobe (2)	Composite	3.92	7.2	0.260
Marglobe (3)	Composite	3.93	7.2	0.295
Bonnie Best	Composite	3.76	7.8	0.265
Average	Core	3.06	6.1	0.259
"	Locule	2.63	5.8	0.057
"	Walls	2.84	5.9	0.289
"	Composite	3.12	6.1	0.269

TABLE III

DEXTROSE AND LEVULOSE CONTENT OF MATURE TOMATO FRUITS
(EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT)

LOCALITY	DEXTROSE	LEVULOSE	RATIO D/L	TOTAL SOLIDS
	per cent.	*per cent.*	*per cent.*	*per cent.*
Burbank	2.06	1.85	1.11	6.9
Chino	2.06	1.71	1.20	7.1
San Fernando	1.93	1.73	1.11	6.6
San Fernando	2.11	1.68	1.25	6.9
San Fernando	2.04	1.81	1.13	6.4
Burbank	2.15	1.84	1.17	6.5
Burbank	2.06	1.92	1.07	6.3
Arlington	1.99	1.91	1.04	6.3
Arlington	1.98	1.92	1.03	6.4
El Monte	2.09	1.97	1.06	6.4
El Monte	1.89	1.59	1.19	6.2
Fruitvale	2.50	2.04	1.22	8.8
Fruitvale	2.37	1.97	1.20	8.4
San Jose	2.35	1.81	1.30	8.1
San Jose	2.24	1.73	1.29	7.9
Isleton	2.39	1.96	1.22	8.5
Palo Alto	2.12	1.78	1.19	8.1
Average	2.14	1.83	1.18	7.2

The average sugar content of tomatoes grown in this state appears to be somewhat higher than that previously reported in the literature.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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RELATION BETWEEN THE GREEN AND THE YELLOW PIGMENTS IN CHLOROTIC LEAVES

(WITH ONE FIGURE)

J. OSERKOWSKY

Willstätter and **Stoll** (**13**) observed that the ratio of green to yellow pigments in green leaves was more or less constant for various plants. **Lubimenko** (**4**) found that the ratio of chlorophyll to carotinoids in many plants varied only a little, but a wider variation was observed between old and young leaves of the same plant; this ratio being, on the whole, larger in the leaves which were richer in chlorophyll. Despite this variation, the data of **Lubimenko** reveal a close relation between the amount of the green and yellow pigments in leaves of different ages. This relation may be approximated by a linear equation. **Sprague** and **Shive** (**12**) and **Schertz** (**8, 9**) determined the chlorophyll content and the yellow pigments in leaves collected at various times during the season. Their data indicate that no consistent quantitative relation exists between the green and the yellow pigments, although in several series of samples a more or less close correlation was found.

A close parallelism between the development of the green and yellow pigments has been observed in etiolated plants exposed to light. **Elfving** (**1**) and **Kohl** (**3**) claimed that the yellow pigments increase in etiolated plants exposed to light. They did not, however, determine the amount of chlorophyll formed during exposure. **Euler** (**2**) and **Sjöberg** (**10**) studied this process quantitatively, and found that on illumination of etiolated seedlings the green and the yellow pigments increase simultaneously.

Chlorosis[1] is usually looked upon as a deficiency of chlorophyll only. Chlorotic leaves are yellow, although severely chlorotic leaves may be a pale cream color or white. Aside from casual observations as to the color of chlorotic leaves, no quantitative data are available in the literature concerning the amount of yellow pigments in chlorotic leaves. The present work was undertaken to ascertain whether there exists a correlation between the amount of yellow pigments and chlorophyll in chlorotic leaves.

The material used in the present investigation was gathered from trees affected with lime-induced chlorosis which was found to be curable by administration of iron salts to the trees. The term "green leaves from treated trees" in table I refers to green leaves from chlorotic trees which became green as a result of treatment with iron salts. Fresh leaves only were used for the analysis of the leaf pigments. The pigments were determined colorimetrically, according to the method of WILLSTÄTTER and STOLL (**14**) as modified by SCHERTZ (**7**). The green color of the potassium chlorophyllin was matched against a similar solution prepared from pure chlorophyll, obtained from fresh green grass by the method of WILLSTÄTTER and STOLL (**13**, pp. 38–41). The yellow pigments in the ether solution were determined colorimetrically against a solution of 0.2 per cent. $K_2Cr_2O_7$, and the results were calculated as "xanthophyll," assuming that all the yellow pigments in the leaves were present as xanthophyll. The calculation was carried out according to the data given by WILLSTÄTTER and STOLL (**14**, p. 25).

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[1] The term chlorosis as used in this paper refers only to chlorosis curable by iron salts.

The methods used in the present work may be open to criticism for the following reasons: The ratio of chlorophyll *a* to chlorophyll *b* may not be the same in green and chlorotic leaves (**6**), and may not be that of the standard chlorophyllin solution. Also the assumption that all the yellow pigment in the leaves was xanthophyll is obviously inaccurate. It is believed, nevertheless, that the data thus obtained afford a means of judging the relative amounts of the yellow pigments present in leaves. It may be noted that the data of WILLSTÄTTER and STOLL (**13, 14**) indicate that xanthophyll predominates over the carotin in green as well as in yellow leaves. Similar results were obtained also by other investigators. The results of the analyses are presented in table I. In figure 1 are plotted the data for Hardy pear leaves only. The data in column 7, table I, show that the ratio of chlorophyll to the yellow pigments is not constant and is smaller in leaves containing the smaller amounts of chlorophyll. But despite the inaccuracies involved in the analytical methods used, the results obtained show a marked positive correlation between the chlorophyll content and the amount of yellow pigments in chlorotic leaves, and in green leaves from chlorotic trees or from trees treated with iron. The coefficient of correlation of chlorophyll to xanthophyll for 31 samples of Hardy pear leaves was found to be 0.934. *Chlorotic leaves are thus deficient in yellow pigments as well as in chlorophyll.* This relation is similar to that existing in etiolated plants. None of the samples analyzed was free from chlorophyll. It is impossible, therefore, to conclude from the available data whether chlorotic leaves devoid of chlorophyll are also devoid of yellow pigments.

Various hypotheses were advanced to explain the more or less constant ratio between the yellow and the green pigments in leaves, which was observed in several instances. LUBIMENKO (**4**) suggested that the yellow and the green pigments of the leaves were derived from a common substance; while SMITH (**11**) concluded from the similarity between the constitution of phytol, a component of chlorophyll, and the carotinoids, that

TABLE I

CHLOROPHYLL AND YELLOW PIGMENTS (REPORTED AS XANTHOPHYLL) IN GREEN AND CHLOROTIC LEAVES

TREES FROM WHICH SAMPLES WERE COLLECTED	PARTS OF TREES FROM WHICH SAMPLES WERE COLLECTED	DESCRIPTION OF LEAVES	DATE OF COLLECTION	PER CENT. OF DRY WEIGHT		RATIO CHLOROPHYLL/ XANTHOPHYLL
				CHLOROPHYLL CONTENT OF LEAVES	XANTHOPHYLL CONTENT OF LEAVES	
Hardy pear	Spurs	Moderately chlorotic	May 27	0.15	0.052	2.9
"	"	Green leaves from treated trees	"	0.78	0.15	5.2
"	"	Moderately chlorotic	June 13	0.12	0.064	1.9
"	"	Severely chlorotic	June 13	0.061	0.032	1.9
"	"	Moderately chlorotic	"	0.18	0.058	3.1
"	"	Green from chlorotic trees	"	0.40	0.083	4.8
"	"	Green from treated trees	"	0.92	0.17	5.4
"	"	Green from treated trees	"	0.78	0.17	4.6
"	"	Severely chlorotic	July 9	0.061	0.028	2.2
"	"	Moderately chlorotic	"	0.18	0.072	2.5
"	"	Green from chlorotic trees	"	0.44	0.10	4.4
"	"	Green from treated trees	"	0.73	0.14	5.2
"	Base of shoot	Severely chlorotic	"	0.071	0.036	2.0
"	"	Slight green from chlorotic trees	"	0.29	0.076	5.1
"	"	Deep green from chlorotic trees	"	0.67	0.15	4.5
"	"	Deep green from treated trees	"	0.77	0.18	4.3
"	Terminal end of shoot	Green from chlorotic trees	"	0.60	0.14	4.3
"	Spurs	Severely chlorotic	July 20	0.10	0.044	2.3
"	"	Moderately chlorotic	"	0.26	0.074	3.5
"	"	Slight green from chlorotic trees	"	0.43	0.13	3.3
"	"	Deep green from chlorotic trees	"	0.96	0.13	7.4

TABLE I—(*Continued*)

Trees from which samples were collected	Parts of trees from which samples were collected	Description of leaves	Date of collection	Per cent. of dry weight		Ratio chlorophyll/ xanthophyll
				Chlorophyll content of leaves	Xanthophyll content of leaves	
Hardy pear	Spurs	Severely chlorotic	Aug. 8	0.057	0.041	1.4
"	"	Moderately chlorotic	"	0.15	0.060	2.5
"	"	Slight green from chlorotic trees	"	0.38	0.10	3.8
"	"	Deep green from chlorotic trees	"	0.65	0.16	4.1
"	"	Deep green from chlorotic trees	"	0.73	0.15	4.9
"	"	Severely chlorotic	Sept. 10	0.081	0.054	1.5
"	"	Moderately chlorotic	"	0.16	0.90	1.8
"	"	Slight green from chlorotic trees	"	0.38	0.10	3.8
"	"	Deep green from chlorotic trees	"	0.66	0.15	4.4
"	"	Deep green from treated trees	"	0.92	0.17	5.4
Bartlett pear	Terminal end of shoots	Severely chlorotic	July 20	0.087	0.047	1.8
"	"	Moderately chlorotic	"	0.27	0.089	3.0
"	"	Slight green from chlorotic trees	"	0.42	0.11	3.8
"	"	Deep green from chlorotic trees	"	0.68	0.12	5.7
Peach	Middle of shoots	Severely chlorotic	"	0.21	0.15	1.4
"	"	Moderately chlorotic	"	0.68	0.20	3.4
"	"	Slight green from chlorotic trees	"	1.10	0.21	5.2
"	"	Deep green from chlorotic trees	"	1.5	0.32	4.7

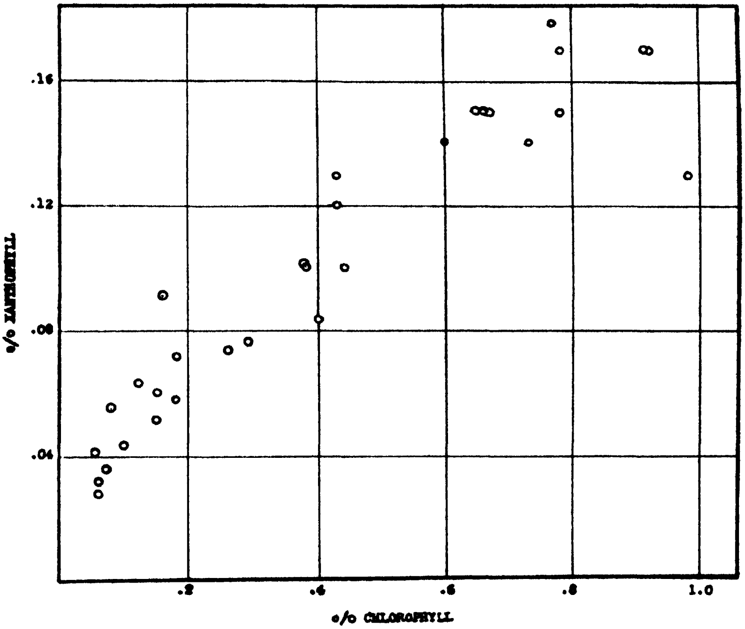

FIG. 1. Yellow pigments (reported as xanthophyll) and chlorophyll in Hardy pear leaves.

"it therefore seems probable that phytol either comes from the carotinoids, forms the carotinoids, or is produced from the same parent substance. In this way the constant proportionality existent between yellow and green pigments of the leaf may be explained" (p. 158).

There is at present no conclusive evidence for any of the hypotheses here mentioned. While any one of them may account for the results reported in this paper, it seems not unlikely that the "active iron" (5), which is the iron compound active in chlorophyll formation, may play a rôle in the formation of yellow leaf pigments.

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UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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A MICRO-METHOD FOR NITROGEN IN PLANT MATERIAL

L. D. DONEEN

Microchemical methods are often desirable or essential for plant nutrition studies. In a recent extensive investigation on nutrition of wheat, samples sufficiently large to determine certain nitrogen fractions of wheat in the seedling and early part of the stooling stage by the usual method were not available, and it became necessary to develop a suitable micro-method.

A review of the literature in the medical field indicated that one of the outstanding methods for determining small amounts of nitrogen is that developed by FOLIN and DENIS (**2, 3, 4, 5, 6**), and later modified by FOLIN and WU (**7**). This method, however, like others reviewed, did not include the fixation of nitrate nitrogen, which is necessary, especially with young plants containing large amounts of nitrate nitrogen. The use of oxidizing agents to promote digestion of organic materials is not new, and is practical for rapid digestion of organic material in the determination of nitrogen (**9, 10, 11, 14**). In some of the more recent works (**1, 8, 9**) protective colloids have been used to stabilize the color obtained by Nesslerization, thus preventing its precipitation with other compounds.

The proposed method involves the use of perchloric acid as an oxidizing agent to aid in the digestion process, and includes the addition of gum arabic as a protective colloid and the conversion of nitrate nitrogen to ammonia.

PROCEDURE.—Place a sample of the plant juice, containing about 1 mg. of nitrogen, in a large test-tube (2.5 × 20 cm.) and evaporate to dryness. (If dry plant material is used, weigh out an equivalent amount.) Add 1 cc. sulphuric acid (nitrogen-free) containing 1 gm. of salicylic acid to 20 cc. of sulphuric acid. Mix thoroughly and allow to stand at least 20 minutes. If large quantities of organic material are necessary to obtain sufficient quantities of nitrogen for Nesslerization, 2 cc. of the sulphuric-salicylic acid mixture may be required. Add approximately 0.3 gm. of sodium thiosulphate and heat the solution gently until fumes appear at the mouth of the test-tube. Cool and add 1 cc. of 60 per cent. perchloric acid to which has been added 0.5 gm. of copper sulphate to 500 cc. of the acid. Digest over covered flame or micro-burner, heating very gently until foaming ceases, and digest at least two minutes after clearing. Usually the digestion mixture becomes clear in five minutes or less.

Transfer the digested mixture to a 100-cc. volumetric flask by washing the test-tube with distilled water and dilute to approximately 50 cc. Add 5 cc. of a 5 per cent. gum arabic solution, and neutralize with 10 per cent. sodium hydroxide. Ordinarily the gum arabic may be used without special preparation, but if color is obtained upon treating it with Nessler's solution, or if reduction to free mercury occurs upon standing for some time, it is necessary to treat the gum arabic as directed by CHILES (**1**).

The results of a series of determinations on identical samples by the proposed micro-method, and the KJELDAHL-GUNNING method modified to include nitrate nitrogen, are given in table I. Samples no. 1 and no. 2 are extract nitrogen or the water-soluble protein nitrogen as obtained by the method of NIGHTINGALE *et al.* (**12, 13**). The other samples represent expressed plant juice. Young plant tissue frequently contains appreciable quantities of nitrate nitrogen, and the wheat plants used in this experiment were known to contain large amounts of it. The results obtained by this colorimetric micro-method check closely with those of the modified KJELDAHL-GUNNING method. Therefore, the proposed method can be used satis-

TABLE I

TOTAL NITROGEN DETERMINED ON PLANT EXTRACTS BY MICRO-METHOD AS COMPARED TO KJELDAHL METHOD

GREEN PLANT TISSUE	SAMPLE NO.	N PER CC.			DIFFERENCE
		MICRO.	AVE. MICRO.	KJELDAHL	
		mg.	*mg.*	*mg.*	*per cent.*
Chrysanthemum leaves, water-soluble fraction	1	0.2745	0.2715	0.2706	+ 0.33
	1	0.2715			
	1	0.2697			
	1	0.2705			
Young wheat plants, water-soluble fraction	2	0.2970	0.2975	0.3057	- 2.68
	2	0.2982			
	2	0.2940			
	2	0.3010			
Young wheat plants, expressed juice	3	1.886	1.874	1.865	+ 0.48
	3	1.851			
	3	1.886			
Seedling wheat plants, expressed juice	4	1.253	1.245	1.246	- 0.08
	4	1.254			
	4	1.245			
	4	1.230			
Bluegrass, expressed juice	5	0.555	0.555	0.565	- 1.76
	5	0.553			
	5	0.551			
	5	0.561			
Cineraria leaves, expressed juice	6	0.819	0.825	0.842	- 2.01
	6	0.831			

factorily to supplement the **Kjeldahl** method when only small samples of plant materials are available for the determination of various nitrogen fractions, including nitrate nitrogen.

The efficiency in converting nitrate nitrogen to ammonia by the proposed method was further determined by additions of nitrate nitrogen to solutions and to plant material. The results given in table II show that approximately 98 per cent. of the nitrate nitrogen added was recovered. It is reasonable to assume, therefore, that nitrate nitrogen naturally present in plant material would be converted likewise.

TABLE II

RECOVERY OF NITRATE NITROGEN ADDED TO WATER AND TO PLANT MATERIAL

SAMPLE	N IN SAMPLE	N ADDED AS NITRATES	TOTAL N DETERMINED	NITRATE NITROGEN RECOVERED	NITRATE N RECOVERED
		mg.	*mg.*	*mg.*	*per cent.*
Distilled water	None	0.0	0.000	0.000	0.0
	"	1.0	0.980	0.980	98.0
	"	"	0.973	0.973	97.3
	"	"	0.984	0.984	98.4
Wheat plant, expressed juice	1.245	0.2	1.444	0.199	99.5
	"	"	1.438	0.193	96.5
	"	"	1.440	0.195	97.5

The method is rapid, since digestion is usually complete in less than 10 minutes. The writer has been able to complete about fifty digestions in an hour by having a number of samples digesting simultaneously.

Division of Agronomy, Soil Section,
Washington Agricultural Experiment Station,
Pullman, Washington.

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STUDIES OF THE HARDINESS OF PLANTS: A MODIFICATION OF THE NEWTON PRESSURE METHOD FOR SMALL SAMPLES[1]

S. T. DEXTER[2]

Introduction

In studying the hardiness of winter wheat, NEWTON (7) outlined a practical test for this quality which involved the measurement of the volume of juice expressed from the hardened leaves of the several varieties. He found that the hardier varieties were more resistant to the pressure treatment and gave a smaller volume of expressed juice than the tenderer ones. MARTIN (3) repeated the work, confirming NEWTON's results in a general way, but finding difficulty in selecting suitable samples. Freezing and subsequent wilting of portions of the plants resulted in a lack of uniformity of samples from day to day. Since rather considerable quantities of leaves were required for pressing, it was found to be impracticable to attempt the selection of uninjured tissue. MEYER (5), working with pitch pine needles, obtained similar results, while STEINMETZ (8) was unable to find any relation between press juice volume and hardiness in two varieties of alfalfa. STEINMETZ, however, froze the alfalfa roots with solid carbon dioxide (about -80° C.) and ground them before pressing.

This paper is devoted largely to the description of a modification of the NEWTON method, which permits the experimenter to use very small samples which may be carefully selected to avoid imperfect leaves. The samples may consist of leaves from single plants, if individual differences are being studied. The weighed sample of leaf blades is placed upon a sheet (or several sheets) of ashless filter paper, so cut that thin layers of the leaves may be folded between sheets of the paper. The circular pad resulting is of such a size that it fits the small press cylinder to be used. The wrapped sample is placed within a washed, sheet-rubber flap to prevent possible contamination or loss of sap. To avoid irregularities of pressure, a pad of several inches of sponge rubber is placed in the bottom of the cylinder. Upon this pad the wrapped sample is placed. Below the piston (above the sample), a large rubber stopper which fits the cylinder is inserted in order to equalize the pressure on top of the sample. Suitable pressures are now applied for a short interval. It is found that the leaves are rarely, if ever, torn or broken within the pad when these precautions are observed.

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[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

[2] National Research Council Fellow.

Since the samples, as from individual plants, are small it is impracticable to try to measure the volumes of expressed sap as was done by NEWTON. In the modification proposed, the juice is taken up completely by the filter paper pads between which the leaves are pressed. The amount of sap expressed is measured in the following way. The unfolded pads and the residual pressed leaves are inserted into test-tubes, and a specified volume of distilled water is added. The sap solutes diffuse from the paper pad into this water. The more mineral matter that is so extracted, the higher will be the electrical conductivity of the water solution. NEWTON (6) has shown that the saps of the various varieties do not differ materially in electrical conductivity. In this way, therefore, one is able to estimate the relative amounts of sap expressed, even though the volumes are very small. There is, however, an additional feature. The leaves of the tender varieties are more bruised, or crushed, by this pressing process than are those of hardy varieties (MARTIN 3). The exosmosis of minerals from such injured tissue might normally be expected to be more or less in proportion to such injury, that is, greater in the case of tender leaves and less in the case of hardy ones. This was found to be the case, even when no clear-cut differences in bruising could be distinguished visually.

The method, as outlined, was repeatedly applied in several ways,—with different sizes of samples, several pressures (66 to 660 atmospheres) and durations of pressures (1 to 5 minutes), and several absorption materials (old linen, cellu-cotton, filter paper). In every case the order of the varieties was correctly indicated, and the tenderer varieties showed higher electrical conductivities than the hardier ones. Measurements made with unhardened tissue did not show this characteristic difference.

Experimental results

On December 1, six 1-gm. samples of perfect leaf blades of Wis. Ped. no. 2 and of Minhardi winter wheat were prepared from plants growing in the field. Each sample was folded into a sheet of ashless filter paper weighing about 1 gm. (Whatman no. 42, 12.5 cm.), placed within a protecting rubber sheet, and subjected to a suddenly applied pressure. Duplicate samples of each variety were pressed at 1000, 2000, and 3000 pounds per square inch respectively. Each sample was pressed in turn, the firmly compacted pad was removed from the press cylinder, completely unfolded, and both paper and sample placed in a 1 × 8″ pyrex test-tube. The tubes were placed in a thermostatically regulated water bath at 2° C., and 25 cc. of distilled water at 2° C. were added to each tube, to cover the sample completely. Conductivity measurements were made after exosmosis had continued for the periods indicated in table I.

TABLE I

SPECIFIC CONDUCTIVITY (× 10^7) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PRESSED WHEAT LEAVES AND THE INCLOSING PADS; MINHARDI AND WISCONSIN PED. 2 VARIETY

PRESSURE	VARIETY	PERIOD OF EXOSMOSIS		
		2 HOURS	6 HOURS	18 HOURS
1000	Minhardi	326	409	549
	Wis. Ped. 2	463	566	799
2000	Minhardi	440	569	740
	Wis. Ped. 2	670	809	1060
3000	Minhardi	571	729	974
	Wis. Ped. 2	774	1117	1343

Minhardi is recognized as a very hardy variety, in field tests, and Wis. Ped. 2 as a hardy variety. From table I one may see that more mineral is obtained, in each case, from the tenderer variety. The injury from the pressing increases with both varieties, with increase in pressure.

On November 15, a similar experiment was run, using duplicate 1-gm. samples of leaves from three varieties of winter wheat, and a pressure of 10,000 pounds per square inch for one minute. The variety, Honor, is a medium hardy variety, tenderer than Wis. Ped. 2. Table II shows the results of this test. Conductivity readings of blanks on unpressed leaves

TABLE II

SPECIFIC CONDUCTIVITY (× 10^7) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PRESSED WHEAT LEAVES AND THE INCLOSING PADS. RESULTS OF EXOSMOSIS FROM FROZEN LEAVES ARE INCLUDED FOR COMPARISON

PERIOD FOR EXOSMOSIS

VARIETY	UNTREATED TISSUES	PRESSED 10,000 LB. PER SQUARE INCH			FROZEN −10° C., 2 HOURS
	2 HOURS	2 HOURS	6 HOURS	20 HOURS	4 HOURS
Minhardi	84	376	486	770	1368
Wis. Ped. 2	81	597	747	1178	2048
Honor	77	623	889	1316	2200
Filter paper	....	....	....	.43	....

and on filter paper are included, as well as the results from exosmosis of leaves frozen at – 10° C. for 2 hours (DEXTER *et al.* **1**, **2**).

The same relations of hardiness and resistance to pressure extraction of sap are seen in this table as in table I. It is interesting to note that the freezing-exosmosis method of DEXTER *et al.* gives conductivity values almost precisely proportional, for the three varieties, to those from the modified NEWTON method. (See also table III.)

In table III are shown the results of an experiment, performed November 27, in which the paper pads and the residual pressed leaves were separately extracted. Trumbull, a somewhat tender variety, and Minhardi were used. A pressure of 3000 pounds per square inch was applied for one minute. Conductivity values of extracts from samples frozen at –10° C. for two hours, on the same day, are included for comparison.

TABLE III

SPECIFIC CONDUCTIVITY ($\times 10^{7}$) (2° C.) IN RECIPROCAL OHMS OF AQUEOUS EXTRACTS OF PAPER PADS ALONE, AND OF PRESSED LEAF TISSUE OF WINTER WHEAT. RESULTS FROM EXTRACTION OF FROZEN SAMPLES INCLUDED FOR COMPARISON

VARIETY	PERIOD FOR EXOSMOSIS							
	PAD ONLY		LEAVES ONLY		TOTAL		FROZEN	
	2 HOURS	6 HOURS	2 HOURS	6 HOURS	2 HOURS	6 HOURS	2 HOURS	6 HOURS
Minhardi	288	299	198	277	486	576	1150	1830
Trumbull	490	490	562	656	952	1146	2340	3870

Table III shows that the varieties are distinguishable on a hardiness basis by extracting the paper pad only, or by extracting the residual leaf tissues. Again the results from the freezing-exosmosis method are comparable with those from the pressure method. With either treatment the tender variety yielded almost exactly double the mineral found in the extract of the hardy variety.

To test another point brought up in the discussion, namely, the question of the soluble mineral matter in the samples, the following experiment was performed. On November 16, eight 5-gm. samples of the entire tops of the plants were prepared from the varieties Minhardi and Trumbull, which were growing in field plats where they had been planted at the usual rates per acre. Of these samples, duplicates for each variety were frozen for two hours at –10° C.; others were heated, in tubes, for 20 minutes on the steam bath; two more pairs were treated with 5 cc. of ethyl ether in closed tubes for an hour; and the last duplicates were thoroughly ground with 5 gm. of washed quartz sand. To each sample, in 1 × 8 pyrex tubes, 50 cc. of distilled water were added and exosmosis was permitted to continue at 2° C. The results of this experiment are shown in table IV.

TABLE IV

SPECIFIC CONDUCTIVITY ($\times 10^6$) (2° C.) OF AQUEOUS EXTRACTS OF WHEAT LEAVES INJURED IN SEVERAL WAYS. EXOSMOSIS CONTINUED 20 HOURS

VARIETY	TREATMENT			
	FROZEN	STEAM BATH	ETHER	GROUND
Minhardi	639	928	695	1120
Trumbull	950	937	730	1173

These results show that while the freezing (or pressure) treatment distinguishes these two varieties very sharply, the other methods used are ineffective. The mineral contents of the extracts of heated, ground, or ether-treated samples do not differ markedly in the two varieties.

Several further experiments were performed to test the influence of the factors more commonly encountered in field samples. It was found that differences in environment, which resulted in changed plant growth, caused variations in the values obtained in these tests. Vegetative plants, grown in well worked soil, were tenderer than smaller, stocky plants grown in firm, compacted soil. Plants shaded half of each day for 8 days were tenderer than adjacent plants receiving full sunlight. When individual plants of a pure line variety were selected for the tests, considerable variation in hardiness was found from plant to plant. When ten plants of Minhardi (very hardy) and ten of Trumbull (rather tender) were so tested, they formed two distinct groups; that is, the hardiest Trumbull plant was tenderer than the tenderest Minhardi plant. In a similar test of Minhardi and Wis. Ped. 2 (both hardy varieties), the groups were by no means distinct, although the average was decidedly in favor of Minhardi, as it should be. All due precaution should be taken in growing plants for these tests, therefore, if the results are to be reliable.

Summary and conclusions

1. A method is described by means of which small samples of wheat leaves can be tested for hardiness according to the principle outlined by NEWTON. The modification of the original method consists in absorbing the expressed juice in ashless filter pads and determining the quantity of sap by measurements of electrical conductivity after extraction of the pad and the residual tissue with distilled water. Extraction of the pad, alone, gives an indication of the relative volume of sap expressed. Extraction of

the pressed tissue, itself, appears to measure the degree of crushing injury in the press. In each case, extracts of tender varieties have higher electrical conductivities than those from hardier plants. The large varietal differences in extraction after pressing are shown to correlate very well with the differences in extraction of frozen samples, according to the method of DEXTER *et al.*

2. It is demonstrated that these differences are not due to correspondingly great differences in "soluble minerals" in the samples. Extraction of plants subjected to freezing or pressure served to differentiate the varieties on a hardiness basis, while extraction of plants injured by heat, ether, or grinding did not.

3. From these facts, it would seem not improbable that a considerable part of freezing injury may be due to the mechanical injury of pressure of the ice formed (MAXIMOV **4**).

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THE OPTICAL LEVER AS A TOOL IN PHYSIOLOGICAL STUDIES

H. A. WADSWORTH

(WITH FIVE FIGURES)

Recent studies in the responses of the sugar-cane plant to varying percentages of soil moisture required the development of a technique for measuring elongation with considerable precision. A modified form of the optical lever, commonly used in physical laboratories for such measurements as the lineal expansion of metals, has been satisfactorily used in this work. So far as is known by the writer, such a device has never before been reported as a tool in plant physiology.

In its modified form the device consists of a small circular mirror mounted normally to a short brass rod which is bifurcated at the end remote from the mirror. This split end is spread to receive the plant member to be under observation. Holes drilled through the two parts of the split end, in a line parallel to the plane of the mirror, permit the pinning of the plant tissue to the end of the lever with a minimum of damage.

The weight of the lever is carried upon a metal rod carrying two projections provided with carefully machined depressions in which two steel pins attached to the mirror support are seated when the device is assembled for use. The lever is so counterpoised that its center of gravity is directly over the line of the pins in order that the plant tissue may be protected from stress when attached to the lever arm.

In use the metal rod is attached to a clamp stand at a convenient height, the mirror pins placed in the depressions, and the plant tissue pinned between the split ends of the lever arm. Vertical displacement of the point of attachment naturally deflects the mirror through an angle θ, while the reflected beam from a fixed source moves through an angle $2\,\theta$.

Observations upon the magnitude of these deflections may readily be made with a laboratory telescope provided with a cross hair and a scale mounted in the plane of the incident and reflected rays. Evidently the apparent magnification of growth depends partially upon the distance of the scale from the mirror. A distance of 1 meter has been found satisfactory with the standard laboratory telescope.

In operation the telescope is clamped horizontally at the height of the center of the mirror and focused upon the image of the scale, which is clamped to the same support as the telescope. Deflections of the mirror after setting cause the reflected beam to move down the image of the scale, while the intersection of the cross hair gives an easily read reference point. With a lever arm of 10 cm. and a distance of 1 meter from the mirror to the scale, a change of 6 cm. per hour in the scale reading has repeatedly been noted with sugar cane. This change of 1 mm. per minute is easily observed by continuous observation.

Figure 1 shows the general assembly of two optical levers measuring the rates of growth of two critical points in a sugar-cane plant. The system is also under gravimetric control in order that some information may be had of soil-moisture conditions. A similar installation with a small pineapple seedling is shown in figure 2. In this case light stirrups of aluminum wire connect the tips of the growing leaves to the lever arms. This system as well as that in figure 1 is under gravimetric control. It should be noted that in each case the balance is securely held in a solid position by excessive weights, except when the occasional weighings are being made.

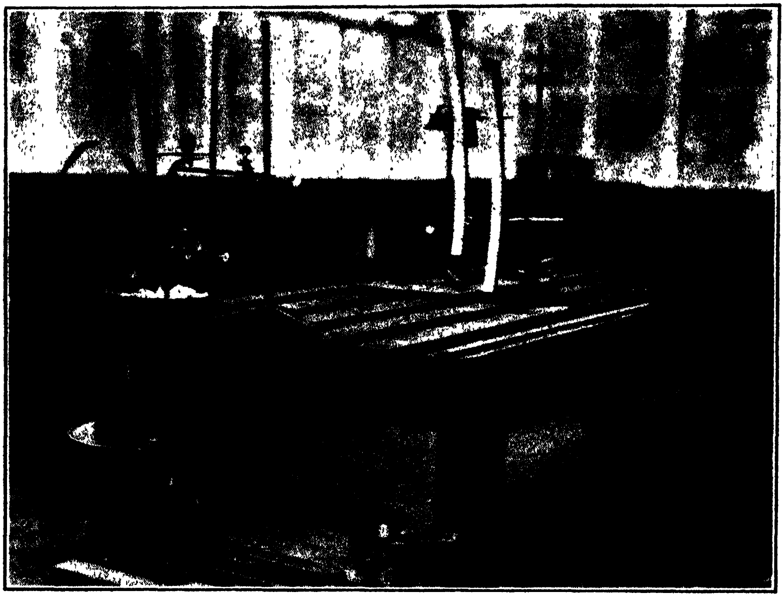

FIG. 1. Two optical levers attached to critical points on a young sugar-cane plant.

Specimen results from these two installations are shown in figures 3 and 4. In figure 3, representing observations with sugar cane, the upper curve shows the vertical displacement of a point on the leaf bundle above the growing point, while the lower curve shows the elongation of the cane itself as measured by the last visible ligule or "dewlap," as it is called locally. This structure presumably is opposite the most active meristematic tissue. The two curves in figure 4 show the growth history of two

FIG. 2. Two optical levers attached to a young pineapple seedling.

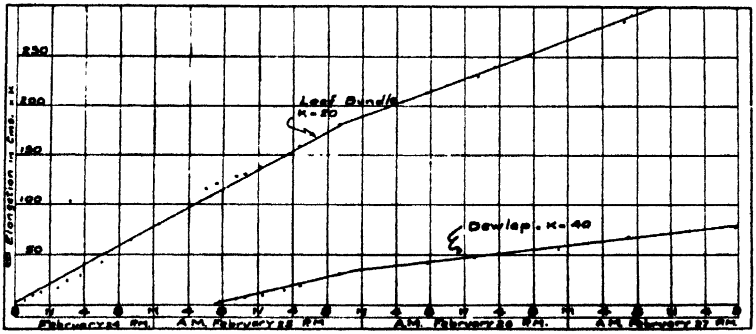

FIG. 3. Growth history with sugar cane as reported by optical levers in figure 1.

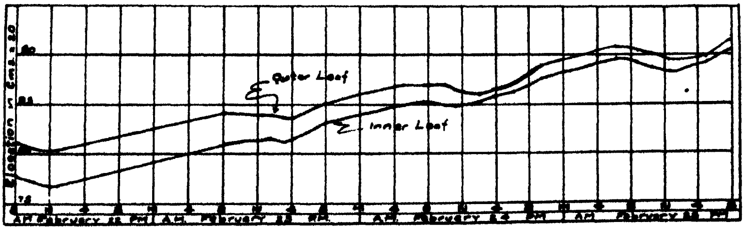

FIG. 4. Growth history of pineapple seedling as reported by optical levers in figure 2.

adjacent leaves in the inner whorl of the pineapple seedling shown in figure 2. The consistent regression during the heat of the middle of the day is interesting; but whether this phenomenon is due to the high temperature in a poorly ventilated greenhouse, to a peculiarity of the individual under observation, or to a general characteristic of the pineapple plant, is not known. The further study of this characteristic and its correlation with environmental factors making for excess rate of loss of moisture from leaf tissue is possible by means of the optical levers.

The geometry of the lever system is a little more complicated than is at first apparent. Figure 5 illustrates a case in which the point under observation moves from P^1 to P^1, the mirror MM and M^1M^1 swinging through the angle $2\ \theta$. The line of sight from the telescope at B bisects the angle KOG. Two scales are shown. One, CBF, is plane, normal to the line of sight and in the plane of the incident and reflected beams. The other, GBK, is in the form of the arc of a circle of radius R and in the same plane as just described.

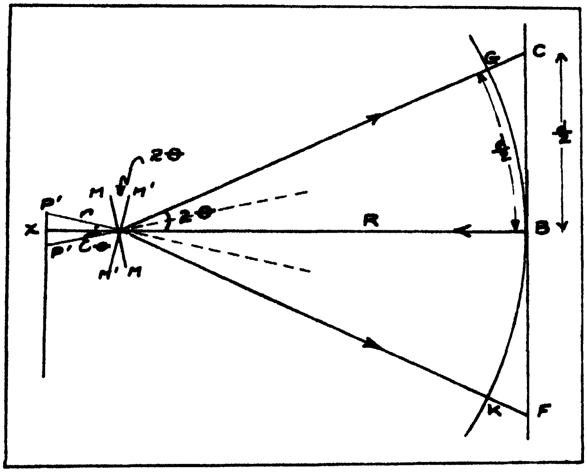

FIG. 5. The geometry of the optical lever.

If the straight scale be used and a direct proportion between the vertical displacement of P and the resulting difference in scale reading is desired, it is evident that the angle θ must be small in order that

$$r \sin \theta = K R \tan 2\ \theta \qquad (1)$$

may express the proportion within the desired degree of precision.

With the curved scale,

$$r \sin \theta = K R\ 2\ \theta \text{ (in radians)} \qquad (2)$$

must express the proportion with the desired precision.

In both cases K is a proportionality constant.

The paper scales normally used for telescopes of this sort are available in 50-cm. lengths. At a radius of 1 meter the subtended angles for a 50-cm. arc is about 28°. Since this angle is twice that between *MM* and M^1M^1, the maximum deflection of the mirror becomes 14° and the maximum value for θ is 7°.

When this value is substituted for θ in (1) and (2), the maximum error, in the case of a flat scale with a total length of 50 cm. and a gross ratio of lever arms of 1:10, is about 2.5 per cent. For the curved scale the corresponding error is about 0.2 per cent. The desirability of the curved scale is obvious, particularly with rapidly growing parts. Attaching the mirror assembly to the supporting rod by means of a clamp and slow motion screw is an obvious improvement which has not as yet been used. This would be of greatest value with such precise work that only the smallest values of θ were permissible.

It is evident, moreover, that the geometry in figure 5 demands that the growing point move vertically. This condition may be closely approached by establishing the point *P* at a considerable distance from any rigidly supported part of the plant.

The writer wishes to express his appreciation of the advice and assistance of Dr. WILLARD H. ELLER, of the Department of Physics, University of Hawaii, in designing the optical levers described.

UNIVERSITY OF HAWAII,
HONOLULU.

CALLUSING AND ROOTING OF COTTON STEM CUTTINGS[1]

H. E. REA

The writer has been interested in the vegetative propagation of cotton for several years and has already made some report of his early experiments with cotton cuttings.[2,3] In the interest of developing further information on this subject, additional experiments were conducted during 1930. Early in the fall a preliminary experiment was completed on a special method of callusing cotton stems. Later a study was made of the relative adaptability of cotton and horticultural plants to propagation by cuttings, in which a large number of these pre-callused cotton cuttings were utilized.

The callusing trials were patterned after an earlier experiment conducted in 1929. In the original trial one lot of 80 cuttings was extremely successful. These cuttings were placed, inverted, in glass jars partially filled with Houston black clay which contained approximately 28 per cent. moisture. After the jars were closed loosely the "butts" of the cuttings were left protruding in the moist atmosphere above the soil surface, for six weeks. Under this condition all except one of the 80 cuttings callused profusely and, compared with the other variations included in the experiment, these cuttings were the most successful. Since the number of cuttings in the original test was small, a similar trial was made in 1930 when 1000 cuttings were used. In the second trial some of the cuttings began to callus in 10 days and in 30 days all of them were perfectly callused.

In previous studies of methods satisfactory for the rooting of cotton cuttings the writer has arbitrarily used the recommendations ordinarily made for the winter propagation of deciduous horticultural cuttings. To test the relative response of cotton cuttings to conditions which are favorable in most respects to horticultural cuttings, 1348 cotton and 1843 horticultural cuttings were placed under identical conditions in a greenhouse propagating bed. As previous experiments with cotton indicated that a relatively high temperature might be more successful, the average temperature maintained for this experiment was somewhat higher than is usual for horticultural cuttings.

The cuttings were placed in rows spaced approximately 2 inches apart and running along the short dimension of the bench. The number of cuttings per row varied inversely as their diameter, and there were usually from

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[1] Contribution from the Division of Agronomy, Texas Agricultural Experiment Station, Technical Series no. 221.

[2] REA, H. E. Asexual reproduction of cotton. Jour. Heredity 19: 357. 1928.

[3] REA, H. E. Callusing cotton stem cuttings. Plant Physiol. 5: 575–586. 1930.

20 to 30 cotton or 30 to 45 horticultural cuttings in each row. The cotton cuttings were placed in alternate rows. Soft, medium mature, and mature wood was used in making the cotton cuttings. The cuttings were collected and prepared identically as described in an earlier paper.[3] The mature cuttings were divided into three groups for separate treatment prior to placement. One group was used without any special treatment while another lot was dipped in warm paraffin and the third lot was thoroughly callused at the time of placing the cuttings in the sand.

The horticultural cuttings used were Amur privet (*Ligustrum amurense*), lilac chaste-tree (*Vitex agnus-castus*), Japanese privet (*Ligustrum japonicum*), Vanhoutte spirea (*Spiraea vanhouttei*), Chinese arborvitae (*Thuja orientalis*), Fortune Forsythia (*Forsythia suspensa fortunei*), and pomegranate (*Punica granatum*). These cuttings were selected from the horticultural plants locally available that ordinarily root satisfactorily under a variety of conditions. All the deciduous horticultural cuttings were made from ripe wood collected just prior to frost and still retaining their leaves. The leaves were removed in making the cuttings. Chinese arborvitae was the only evergreen used and these cuttings were made in the usual manner.

Separate lots of both cotton and horticultural cuttings were set in beds of quartz and granite sand, with and without a peat substratum. After the cuttings were placed, a glass sash covering was provided for most of them. Bottom heat was supplied by means of hot water pipes under the benches and the general temperature of the greenhouse was controlled by an atmospheric thermostat set at 75° F. The temperature recorded during the experiment averaged 78° with a normal variation of 10° F.

The results secured with cotton cuttings as presented in table I show that only 8.2 per cent. of the 1348 cuttings used were successfully rooted. Over 200 cuttings each of the soft and medium mature cotton cuttings were tried without getting a single cutting to strike root. However, the three groups of mature wood cuttings rooted in excess of 10 per cent. Cotton cuttings made from mature wood immediately before using and without any special treatment gave a rooting percentage of 11.3. The pre-callusing of the cuttings was of little value as only 10.8 per cent. of that group rooted. Dipping the mature cuttings in paraffin did increase the percentage rooted to 15.3.

The results of the three variations in treatment provided for each type of cotton cutting failed to indicate any very promising method of rooting these cuttings. Heretofore the quartz sand locally available has frequently been used in attempts to root cotton. This sand invariably contains small quantities of clay and as this is generally regarded as undesirable as a cut-

TABLE I

PERCENTAGE OF COTTON AND HORTICULTURAL CUTTINGS ROOTED

TYPE OF CUTTING	NO. OF CUTTINGS USED	AVERAGE OF ALL TREATMENTS	MEDIUM USED		SUBSTRATUM		COVERING	
			QUARTZ SAND	GRANITE SAND	NONE	PEAT	NONE	GLASS SASH
Cotton stem cuttings	1348	8.2	10.0	6.5	10.1	6.6	10.1	7.3
Soft wood	235	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Medium mature wood	232	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Callused mature wood	325	10.8	16.6	4.9	13.9	7.5	11.8	10.2
Mature wood	230	11.3	6.3	14.0	15.8	6.8	12.2	10.9
Paraffin mature wood	326	15.3	19.1	11.8	15.6	15.1	20.2	12.6
Horticultural cuttings	1843	31.0	36.0	25.5	31.3	31.7	17.3	30.9
Amur privet	168	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Japanese privet	275	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vanhoutte spirea	449	26.1	31.9	19.8	22.3	29.8	16.3	30.8
Chinese arborvitae	353	39.7	41.0	38.3	41.6	37.8	19.5	49.2
Pomegranate	281	47.7	64.4	28.8	56.8	37.6	——	47.7*
Fortune Forsythia	147	51.7	62.0	39.7	44.0	59.7	——	51.7*
Lilac chaste-tree	170	61.3	62.4	59.7	60.9	61.4	63.8	58.9

* Not used in the comparison of covered and exposed cuttings.

ting medium, a pure granite sand was used[4, 5, 6]. However, only 6.5 per cent. of the cotton cuttings placed in the granite sand rooted compared with 10.0 per cent. of those in the quartz sand. Some horticultural propagators have used mixtures of peat moss as a medium with unusual success[7, 8, 9]. In the present experiment a substratum of peat moss was used under the sand in some sections of the cutting bed. This was tried in comparison with sand alone with the result that only 6.6 per cent. of the cotton cuttings with peat moss under them rooted while 10.1 per cent. of those without it rooted. Good results have been reported from some horticultural cuttings by covering the cutting bed with a glass sash[4, 5]. In this experiment a portion of the cuttings was under sash. The cotton cuttings under the sash rooted to the exent of only 7.3 compared with 10.1 per cent. for those exposed.

The results secured with horticultural cuttings are also presented in table I and show that 31.0 per cent. of the 1843 cuttings used were rooted. Although this was low for this class of cuttings it was twice as high as the best result secured from any group of cotton cuttings. Also this percentage was made in face of the fact that none of the Amur or Japanese privet cuttings rooted.

Amur and Japanese privet are both very hardy plants and usually their cuttings root even though conditions are not altogether favorable. In this experiment, however, over 100 cuttings of each of these plants were used without obtaining a single rooted cutting. An investigation of the situation indicated that the Amur and Japanese privet cuttings were killed by excessive bottom heat. In making these cuttings they were cut an inch or two longer than the others and were unintentionally placed deeper.

All of the other horticultural cuttings rooted more frequently than did any group of the cotton cuttings. Lilac chaste-tree gave the highest proportion of rooted cuttings, as 61.3 per cent. of them were successful. Also roots were produced by 51.7, 47.7, 39.7, and 26.1 per cent. of the Forsythia, pomegranate, arborvitae and spirea cuttings, respectively.

Without an exception the horticultural cuttings rooted more frequently in the quartz sand than in the granite sand. On the average, 36.0 per cent. of these cuttings rooted when placed in quartz compared with 25.5 per cent.

[4] BAILEY, L. H. The nursery book. 19th ed. Macmillan Co. pp. 44–58. 1914.

[5] HOTTES, A. C. Practical plant propagation. A. T. De La Mare Co. pp. 61–85. 1917.

[6] YERKES, GUY E. Propagation of trees and shrubs. U. S. D. A. Farmers' Bull. 1567. 1929.

[7] ESPER, H. C. The effect of time of taking, medium, and bottom heat on the rooting of evergreen cuttings. Ohio Agr. Exp. Sta. Bimonthly Bull. 154: 9–17. 1932.

[8] HITCHCOCK, A. E. Investigations in propagation. So. Florist & Nurseryman 27: no. 13. 12, 27. 1929.

[9] REUDEL, FRANKLIN J. Methods of propagation. So. Florist & Nurseryman 25: no. 17. 8–9. 1928.

in granite sand. The proportion of horticultural cuttings rooting with and without a peat moss substratum were 31.7 and 31.3 per cent. respectively. Although there was little difference in the composite results, the use of peat moss appeared to have increased the percentage of rooted cuttings for spirea and Forsythia. On the other hand, the pomegranate cuttings were injured by peat moss while chaste-tree and arborvitae cuttings rooted just as readily whether peat moss was used or not. Undoubtedly the horticultural cuttings were favored by the use of the glass sash. Since none of the pomegranate or Forsythia cuttings was exposed, their records were eliminated from this comparison. The average results of the other horticultural cuttings show that 30.9 and 17.3 per cent. of the covered and exposed cuttings rooted, respectively. However, the chaste-tree cuttings rooted satisfactorily whether covered or exposed.

The main results are briefly summarized.

1. The results obtained with cotton cuttings confirm the results on callusing obtained previously.
2. Under identical conditions, the proportion of cotton cuttings striking root in these experiments was markedly lower than for lilac chaste-tree, Fortune Forsythia, pomegranate, Chinese arborvitae, or Vanhoutte spirea.
3. The selection of mature wood is important in the winter propagation of cotton cuttings. The soft and medium mature cotton cuttings used in his experiment were worthless.
4. The pre-callusing of mature cotton cuttings failed to prove of value but dipping the cuttings in paraffin increased the percentage of rooting slightly.
5. The highest percentages of rooting with mature cotton cuttings were secured in an exposed greenhouse bed of quartz sand free of any substratum.

TEXAS AGRICULTURAL EXPERIMENT STATION,
SUBSTATION NO. 5,
TEMPLE, TEXAS.

BRIEF PAPERS

RESPONSE OF IRIS TO SOIL REACTION

(WITH TWO FIGURES)

Recommendations in popular literature vary considerably as to the optimum soil reaction for the Bearded iris, *Iris germanica*, ranging from "acid" to "alkaline." DYKES[1] states that "The Bearded Irises all, without exception, want dry conditions and the only hope in damp situations is to make raised beds for them. They also need lime in the soil and this is, in most cases, best supplied in the form of old mortar rubble. The Apogons do not, as a rule, want lime, except possibly the Spurias and some, such as the Californians, will not grow in a soil that is highly charged with lime."

Since soils on which the iris is grown in Ohio vary widely in reaction, it seemed desirable to determine the optimum condition or range for them. A series of plots on a raised bed in the greenhouse, which had already been prepared for studying other species of flowers, was used for the purpose.[2] These plots were 7 × 8 feet in size and were filled with Canfield silt loam, in June, 1929. The pH value of the soil was 5.0 at the time it was moved into the greenhouse. A one-inch layer of German peat (pH 3.5) was added in an endeavor to improve the humus content. Increments of aluminum sulphate or hydrated lime were added to the plots until a range of pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 was attained for the eight plots respectively. Readings of the pH value of each plot were made biweekly, either electrometrically or by means of the La Motte soil tester.

On September 1, 1930, irises were planted in these plots, using Alcazar and another German variety, the name of which was not determined. The rhizomes were approximately the same size at the time of planting, but the weights were not recorded. Three plants of each variety were set to the plot and they received identical care with the exception of occasional additions of aluminum sulphate or hydrated lime as was necessary to maintain the desired reaction of the soil.

For the first four months no appreciable differences were noted in the growth or color of the plants. Beginning January 1, 1931, a darker color and larger size of foliage were noted in the plants growing on plots 7 and 8 (pH 7.5 and 8.0 respectively). Plots 1 and 2 by this time showed a somewhat stunted appearance, with some tip burn. The succeeding plots were increasingly better, particularly in the alkaline ones, as noted. Throughout the following eighteen months there was a gradual widening of the growth behavior between plots 1 and 8 until those in the former were practically worthless. Whether this injury to the plants at pH 4.5 and 5.0 was due to the high concentration of hydrogen ions or to toxicity due to soluble aluminum was not definitely determined, but probably the latter played an important rôle.

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[1] DYKES, W. R. Handbook of garden irises. Hopkinson. 1924. p. 235.

[2] WIGGIN, W. W., and GOURLEY, J. H. Studies on the reaction of greenhouse soils to the growth of plants. Ohio Agr. Exp. Sta. Bull. 484. 1931.

Figure 1 shows a typical sample of leaf from each plot. There is a fairly consistent gradient in size, color and amount of tip burn from plot 1 to plot 8.

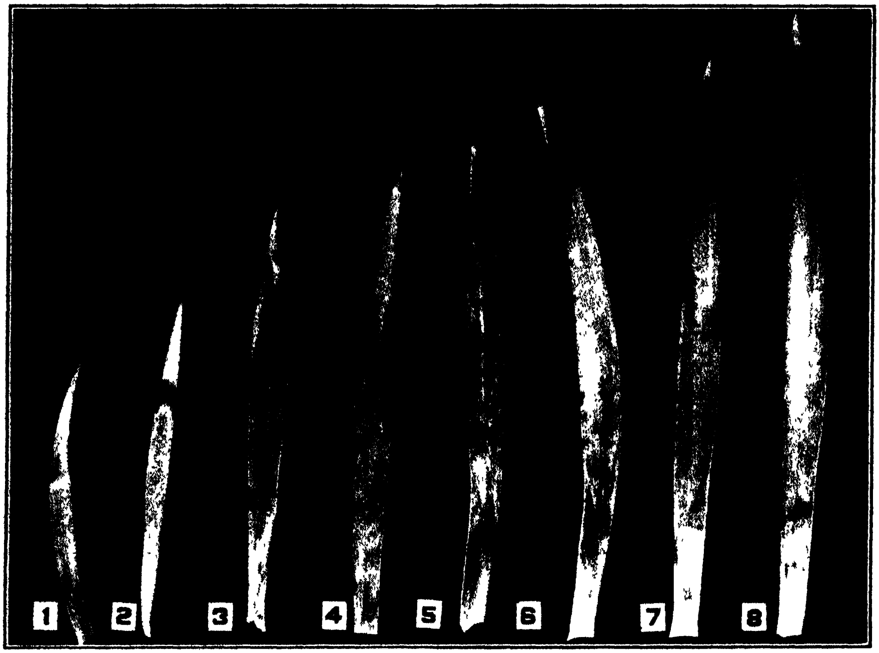

FIG. 1. A typical leaf from soil reaction plots having a range of pH 4.5 to 8.0.

Figure 2 shows a typical plant from each plot, photographed June 15, 1931.

At the time of removal from the bed, July 22, 1932, the Alcazar plants were weighed and the length of typical leaves measured. With one slight exception, plot 5, the weight is in an ascending order from plot 1 to 8, increasingly higher as acidity decreases; that is, from plot 1 to 8. The length of flower stems was measured on June 15, 1931, and, with other data, are shown in table I.

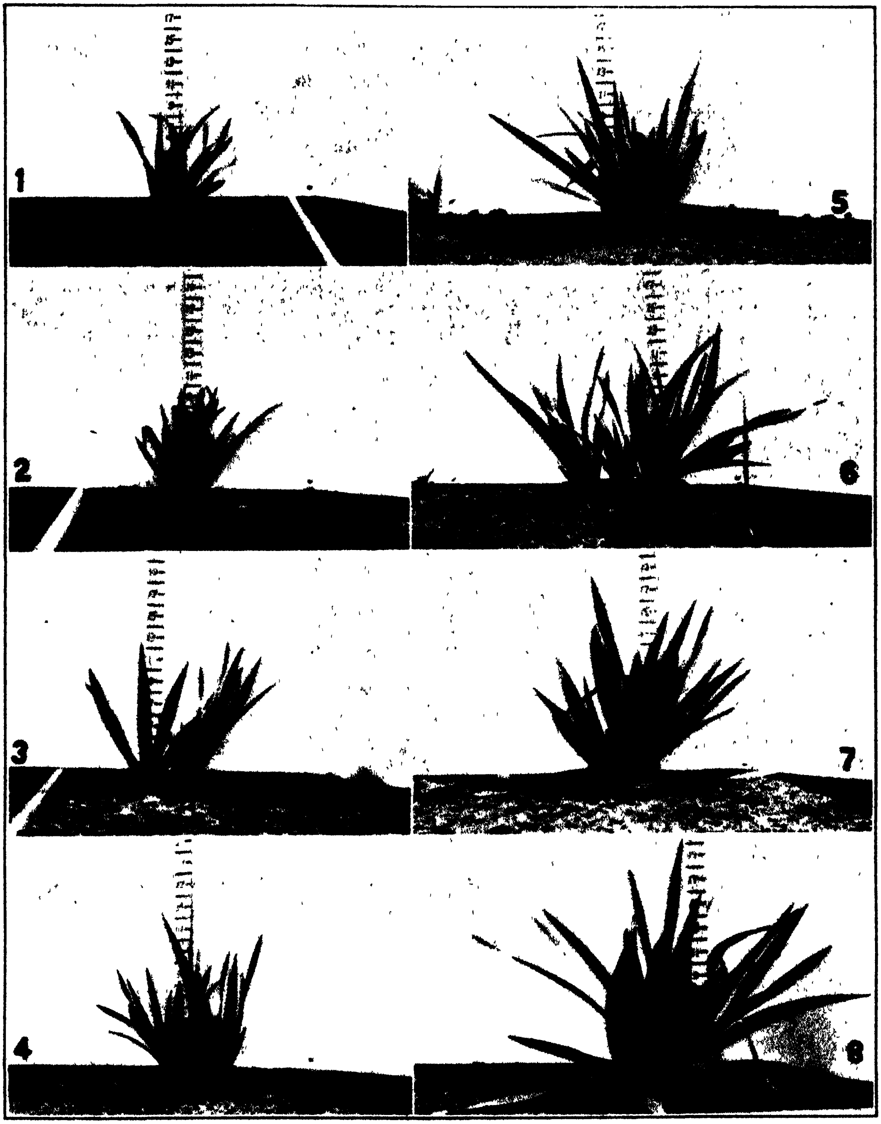

FIG. 2. Alcazar iris plants typical of those grown in soil reaction plots from pH 4.5 to 8.0.

From this work it appears that the German iris, Alcazar, thrives much better when the soil reaction is nearly neutral to alkaline than in acid soils. Since these plants were kept rather dry throughout to prevent injury, the matter of wet soil is eliminated. The conclusion may be drawn that iris

TABLE I

SOME EFFECTS OF SOIL REACTION ON GROWTH OF ALCAZAR IRIS PLANTS, SEPT. 1, 1930, TO JULY 22, 1932

PLOT	RE-ACTION	AVERAGE WEIGHT OF 3 PLANTS	AVERAGE NUMBER OF SHOOTS PER PLANT	AVERAGE HEIGHT OF FOLIAGE	LENGTH OF FLOWER STEMS, JUNE 15, 1931	CONDITION OF FOLIAGE
	*pH**	*gm.*		*inches*	*inches*	
1	4.49	276	4	9	3	Very pale, tip burn
2	4.86	316	4	11	5	Very pale, tip burn
3	5.46	385	6	13	8	Pale, tip burn
4	6.05	401	5	14	10	Pale, tip burn
5	6.48	393	5	15	10	Green
6	6.93	529	5	16	14	Dark green
7	7.56	561	4	18	16	Dark green
8	8.05	677	5	20	22	Very dark green

* The last reading before the iris plants were removed, recorded electrometrically with a quinhydrone electrode, June 21, 1932.

plants growing on acid soils may be improved if the soil is treated with lime until the reaction is nearly neutral or above.—J. H. GOURLEY, *Ohio Agricultural Experiment Station, Wooster, Ohio.*

GROWTH OF TREE SEEDLINGS IN RELATION TO LIGHT INTENSITY AND CONCENTRATION OF NUTRIENT SOLUTION[1]

(WITH ONE FIGURE)

The importance of available mineral nutrients in the shade tolerance of trees has been emphasized by LUNDEGARDH,[2] who points out that the presence of abundant mineral nutrients in the soil favors chlorophyll and foliage formation and thus increases the assimilative capacity of plants under shade conditions. Sufficient experimental data relative to the effects of mineral nutrients on plants grown at low light intensities are lacking, however. The following experiments were carried out to determine the effects of concentration of mineral nutrients on tree seedlings grown at or near the minimum light intensity required for growth.

[1] The author wishes to express his appreciation to Dr. R. B. HARVEY of the University of Minnesota for his encouragement and suggestions during the course of the experimental work and for the material assistance and facilities offered by the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station.

[2] LUNDEGARDH, H. Klima und Boden in ihrer. Wirkung auf das Pflanzenleben. Jena. 1925.

Seedlings of *Fraxinus pennsylvanica* were germinated in quartz sand and then transferred to nutrient solutions of the same composition but varying total concentrations. The solution used was a three-salt solution, consisting of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, corresponding in molecular proportions to solution R4S1 of the National Research Council Bulletin on "A plan for cooperative research on the salt requirements of representative agricultural plants."[3] Iron was added in the form of ferric phosphate in the concentration of approximately 3 parts per million. Three concentrations of solution were used corresponding to 1.0, 0.1, and 0.01 atmospheres, and the solutions were renewed at four-day intervals during the growing period.

The seedlings were grown in four light intensities, obtained by use of electric lights and corresponding to 130, 70, 48, and 31 foot-candles as measured with the Macbeth illuminometer. Previous experimentation had indicated that the minimum light intensity for growth was approximately 50 foot-candles when using continuous illumination. Temperature was kept at 70° F. with a maximum variation of about 2 degrees. Relative humidity was maintained at approximately 80 per cent.

At the end of a ten-week growing period the seedlings were removed from the jars and compared as to gain in dry weight, length of roots and

TABLE I

COMPARISON OF THE GROWTH AND DEVELOPMENT OF *Fraxinus* SEEDLINGS IN THREE LIGHT INTENSITIES AND THREE CONCENTRATIONS OF NUTRIENT SOLUTION

LIGHT INTENSITY	SOLUTION CONCENTRATION	GAIN IN DRY WEIGHT*	LENGTH†		WATER TRANSPIRED PER GM. DRY WEIGHT GAINED
			TOPS	ROOTS	
foot candles	*atm.*	*gm.*	*mm.*	*mm.*	*gm.*
130	1.00	3.20	65	218	370.4
130	0.10	3.27	72	150	439.4
130	0.01	2.43	61	131	374.5
70	1.00	1.70	39	175	498.1
70	0.10	1.74	51	119	
70	0.01	1.50	49	117	
48	1.00	0.84	53	120	583.9
48	0.10	0.86	58	107	971.6
48	0.01	0.77	51	100	843.2

* Average of 24 individuals.
† Average of 39 individuals.

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[3] LIVINGSTON, B. E., CROCKER, WM., and KELLERMAN, K. F. A plan for cooperative research on the salt requirements of representative agricultural plants. Baltimore. 1919.

stems, general vigor, and the amount of water lost in transpiration. The results are summarized in table I and figure 1 and refer to the seedlings grown in the three higher light intensities since the plants grown in the lowest light intensity did not survive more than one or two weeks in any of the nutrient solutions.

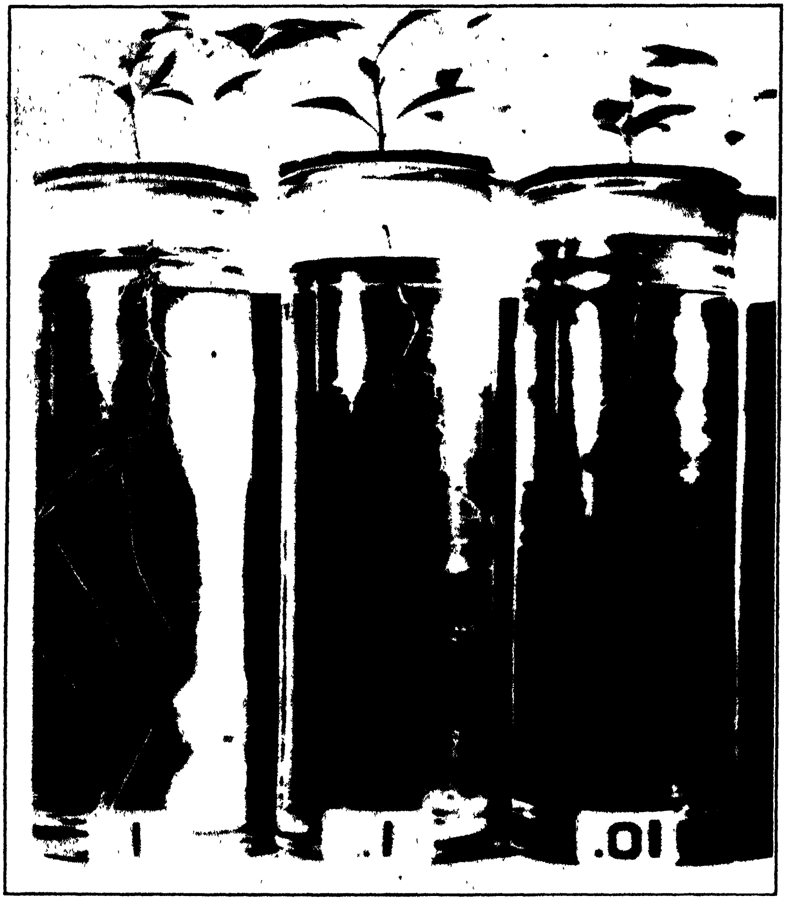

FIG. 1. Photograph showing effect of concentration of nutrient solution on type of root growth.

The data obtained, relative to the effect of concentration of nutrients on light requirements of the tree seedlings used, indicate that while a sufficient supply of nutrients must be present to satisfy the needs of the plant, the minimum light requirement cannot be lowered by increasing the amount of available nutrients. The length of the period of survival for the seedlings grown under a sub-minimum light intensity was the same in all cases regardless of the solution concentration.

The greatest response to an increase in concentration of the nutrient solution occurred at the higher light intensities, indicating a greater need for nutrients under those conditions. Thus increasing the concentration of solution from 0.01 to 0.1 atm. for a light intensity of 48 foot-candles resulted in an increase in dry weight of 11.7 per cent. For seedlings growing under light intensities of 70 and 130 foot-candles there resulted increases of 16.0 per cent. and 34.5 per cent. respectively.

The marked increase in total length of roots developed in a solution of 1.0 atm. concentration over those grown in a solution of 0.1 atm. was more than offset by a greater abundance of lateral branching at the lower concentration. The effect of concentration on type of root growth is illustrated in figure 1.

The water requirement, expressed in terms of grams of water transpired per gram of dry material gained, might seem to indicate a greater need for water at the lower light intensities. This, however, was due to a reduction in the rate of assimilation of material rather than to a high intake of moisture.—GEORGE P. STEINBAUER, *Minnesota Agricultural Experiment Station, St. Paul, Minnesota.*

METHODS OF MEASURING STRAWBERRY LEAF AREAS

In MILLER'S[1] recent text on plant physiology, five methods of measuring the leaf surface of plants are given. In the writer's work with strawberries during the past year, however, three other methods were used, none of which was mentioned in this text. As these methods were adopted as more useful under the conditions encountered for field and greenhouse work than those given by MILLER, it seems worth while to call attention to them. The first and second are not new but apparently are not as well known as they should be. The third is apparently little known. They are (1) the leaf-product, (2) the matching, and (3) the adjustable platform-planimeter methods.

LEAF-PRODUCT METHOD.—This method, used by LIVINGSTON and associates,[2] is based on the correlation between extent of leaf surface and lineal measurements of the leaves. It provides an index of the actual area of varying degrees of accuracy. The writer[3] has used the following

[1] MILLER, E. C. Plant Physiology. McGraw-Hill Co. 1931.

[2] McLEAN, F. T. A preliminary study of climatic conditions in Maryland, as related to plant growth. Physiol. Res. 2: 129–208. 1917.

[3] DARROW, GEO. M. Experimental studies in the growth and development of strawberry plants. Jour. Agr. Res. 41: 307–325. 1930.

indices in actual practice according to the degree of accuracy required: length of middle leaflet; length × breadth (leaf product) of middle leaflet; and length × breadth of all three leaflets of the strawberry leaf. These indices were accurate to within 5 per cent., the single measurement of length being least accurate; the leaf-product (length × breadth) of the middle leaflet more accurate; and the sum of the leaf-products of all three leaflets the most accurate index of actual area. The accuracy of this index is obviously dependent on (1) the number of measurements used to obtain a factor to translate leaf length or length × breadth into area; (2) whether the measurements obtained are for one or for several varieties; and (3) the uniformity of the conditions under which the plant material is grown. Under this method may be listed that of CARUTHERS,[4] in which breadth was used as an index of leaf area of *Ribes* much within an accuracy of 5 per cent.

MATCHING METHOD.—This method has been used by several investigators, but was described to the writer by C. F. KINMAN, of the Division of Horticultural Crops and Diseases, who used it to obtain the area of great numbers of leaves of pears and other fruits. For this a pasteboard form equal to the largest leaf encountered is cut out, its area measured with the planimeter and the size written on the form. On this form are drawn outlines of successively smaller leaves, each of which is measured and the area recorded on the form corresponding to each. Leaves on trees or in the laboratory are matched rapidly and with an accuracy (table I) approaching or equal to that when a planimeter is used. The more uniformly shaped leaves are measured with the greatest accuracy and rapidity (several hundreds per hour) by this method. Even actual areas of leaves of many plants can be obtained almost as quickly as length alone can be measured. The matching of strawberry leaves, however, is difficult as compared with matching of leaves of some other plants; yet it has been possible to follow the increase and decrease in leaf area of hundreds of experimental plants in the field and greenhouse with great accuracy and with relative ease. The use of several pasteboard forms of different sizes fastened to heavy cardboard strips (8 small, 6 medium, and 4 large in our work) was found to be more than 20 per cent. faster than a single form with concentric outlines of smaller leaves on it. If very accurate data are wanted, forms for each variety must be used; otherwise a set of forms for all varieties can be used.

ADJUSTABLE PLATFORM-PLANIMETER METHOD.—This method was suggested by W. M. ATWOOD, of the Oregon State College. To one edge of an adjustable stand used for camera lucida drawings a piece of automobile celophane is so fastened by means of thumb tacks that a leaf can be inserted under the celophane but on the stand. The stand, quickly adjustable to any angle in any direction, is used to hold the leaf and the planimeter for measurements of actual areas. Leaves on shrubs and herbs within easy reach of the ground can quickly be measured. Such a stand was found especially useful when measuring leaves of experimental strawberry plants in the greenhouse.

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[4] CARUTHERS, R. S. A scale for measuring areas of *Ribes* leaves. Phytopath. 19: 399–405. 1929.

RELATIVE SPEED AND ACCURACY.—The relative speed and accuracy of the three methods and of variations of each were calculated for one variety, O. S. C. 192 (Marshall x *Fragaria chiloensis*). In a first test of speed and accuracy, 10 leaves were used to obtain correction factors and these applied to measurements of 18 leaves (54 leaflets). In the second test 50 leaves were used to obtain correction factors and these applied to measurements of 50 other leaves. Results of both tests are given in table I.

TABLE I

COMPARISON OF SPEED AND ACCURACY OF METHODS FOR OBTAINING LEAF AREAS OF STRAWBERRIES

	PLANIMETER	MATCHING	LENGTH	LENGTH x BREADTH
No. leaves per hour				
First test	48	156	136	114
Second test	21	172	172	108
Percentage of speed				
First test	31	100	87	73
Second test	21	100	100	63
Percentage of error				
First test	0.0	3.2	4.3	4.7
Second test	0.0	1.8	4.3	0.2

As to speed, the results were surprising even to those using the methods. The matching method was, on the whole, faster than that of measuring length only, and was considerably more accurate. When 50 leaves were measured as in the second test, the accuracy of the length x breadth method was 0.2 per cent. and of the matching method 1.8 per cent. It is probable that under ordinary conditions either the matching or length x breadth methods are as accurate as using the planimeter itself. It should be remembered that in these tests the use of 50 strawberry leaves meant measuring 150 leaflets on the plants, and that with plants having simple leaves the number measured per hour should be at least three times as great as with the strawberry.—GEORGE M. DARROW, *U. S. Department of Agriculture, Washington, D. C.*

NOTES

Atlantic City Meeting.—The ninth annual meeting of the American Society of Plant Physiologists will be held at Atlantic City, New Jersey, during convocation week, December 27–31, 1932. The headquarters for the botanical societies is the Traymore Hotel. Announcements regarding railway fares, hotel rates, etc., will be published in Science. Close to the great eastern centers of population, Atlantic City offers opportunity for a large gathering. The meeting will be notable for the SACHS centennial celebration, and for the third award of the STEPHEN HALES prize. Members of the Society are urged to include the annual dinner in their plans, as the life membership award and the STEPHEN HALES award are both usually announced at the annual dinner. In the past the banquet has been one of the most pleasant features of the entire meeting.

Summer Meeting.—The recent summer meeting at the University of Wisconsin, which was held jointly with the corn belt section of the American Society of Agronomy, July 11–13, 1932, was greatly enjoyed by those who were able to attend the various sessions and share in the profitable laboratory and field demonstrations. The attendance of plant physiologists was estimated roughly at about 40, and the total attendance at about 180. Of special interest to the physiologists was the exhibition of portraits of plant physiologists which have been collected during recent years by Dr. R. B. HARVEY of Minnesota. In connection with the exhibition HARVEY gave an illustrated lecture on his experiences among the Russian people several years ago. Much appreciated, also, was the interesting discussion of the "Relations between plant composition and growth responses" by Dr. H. R. KRAYBILL of Purdue University.

The laboratory demonstrations of experimental work on hardiness, photosynthesis, nitrogen fixation, soil fertility testing, corn seed drying, etc., were very attractive. The field trips were used to demonstrate such matters as disease control measures, hybridization and selection of various crops, and cutting practices with reference to the permanence of alfalfa stands.

The boat trip on Lake Mendota was a delightful experience for those who were able to take it. The meetings were decidedly effective, and everyone enjoyed the splendid fellowship and cordiality of the occasion.

Syracuse Meeting, A. A. A. S.—The Syracuse meeting of the A. A. A. S. and affiliated societies was small, but made up in genuine enjoyment what it lacked in numbers. Indeed, mere size has become a detriment to good meetings. Few papers were read, a decided improvement over the winter arrangements. The main botanical addresses were by Dr. R. H. TRUE and Dr. W. L. BRAY, and there was a symposium on cell wall chemistry. The main entertainment was in the form of field trips, and these were planned and handled in excellent fashion. No one who went on the delightful excursion to the plunge-basin lakes of the Jamesville region, and to the home of *Scolopendrium vulgare,* will ever forget it; nor the all-day trip to the marl and peat bogs of West Junius; nor the afternoon at the salt flats near Lake Onondaga. If ever in doubt as to the most appropriate thing for a summer meeting, plan an interesting field trip. Nature yields a symposium suited to all tastes, and she never talks too long!

Julius von Sachs.—The hundredth anniversary of the birth of JULIUS VON SACHS is at hand. Born October 2, 1832, we are happy to honor his memory in 1932. During the year PLANT PHYSIOLOGY has carried portraits of the peerless organizer of this field of Botany, and photographs of his famous laboratory at Würzburg. It is with great pleasure that we present with this issue an unpublished photograph of SACHS at the age of about 40 years (1872), with several of his students. Seated at the right of SACHS is HUGO DE VRIES. Standing behind him is JOSEF SCHUCH, and at the right, standing, is J. BARANETZKY. It is an exceedingly interesting group, and emphasizes the inspirational leadership of SACHS during that period of botanical development. Botanists, and particularly plant physiologists, are greatly indebted to SACHS, whose ability as an investigator and teacher places him in the front rank of the late 19th century scientific leaders. May the plant physiologists of this country follow worthily but humbly in his footsteps, to build an appropriate superstructure upon the immortal foundations which he laid for us.

Incorporation.—Without the slightest disturbance to indicate the transpiring of important events, the old American Society of Plant Physiologists went out of existence, and a new society was born. The incorporators of the American Society of Plant Physiologists met at 4:20 P. M. on July 5, 1932, in room 342, West Wing of the U. S. Department of Agriculture Building, Washington, D. C. Those who met were Dr. P. F. CULLINAN, Dr. ANNIE M. HURD-KARRER, Dr. CHARLES F. SWINGLE, Dr. B. C. BRUNSTETTER, and Dr. JOHN W. ROBERTS. Dr. CULLINAN was elected temporary chairman, and Dr. ROBERTS, temporary secretary.

"It was moved, seconded, and passed by unanimous vote, that all the present existing members of the unincorporated society be elected members of the incorporated society, with their present rights and privileges."

"It was moved, seconded, and passed by unanimous vote, that the meeting adjourn till the next regular meeting of the American Society of Plant Physiologists." (Signed) JOHN W. ROBERTS, temporary secretary.

With these simple rites the Society begins its corporate existence. The way is open now for larger opportunities and larger responsibilities. A solid foundation has been laid, upon which the future development can securely rest.

Treasurer's Report.—During the last fiscal year the financial strength of the Society was put to its severest test so far. It became necessary, because of the earlier issuance of the quarterly numbers, to pay for five issues in a single fiscal year. The additional cost was about $1000. In spite of this extraordinary expense, the assets of the Society on July 1 were as great as they were a year ago, which means that even in a year of severe depression we have been able to meet this strain without resorting to any financial expedients. This fact should be a source of great satisfaction to all officers and friends of the Society. In addition to this cheering situation, the BARNES Life Membership fund has reached a total of $2105, within $400 of the amount required to finance this fund permanently. The STEPHEN HALES prize endowment is $1100, and there is $164 in the general endowment fund. There are, besides these funds, reserves and moneys on deposit that bring the total assets in cash and bonds to over $8000. At the same time, unsold volumes of PLANT PHYSIOLOGY are held to a value of over $2000. This report shows marvelous progress for a struggling young Society that started publication in 1926 with nothing but faith in the worthwhileness of the tasks which were being undertaken. With the extraordinary strain of the finances of 1931–32 behind us, and with watchful care of the expenditures for publication, it is hoped that the next treasurer's report may indicate a resumption of financial growth.

Plant Physiology.—During the last year PLANT PHYSIOLOGY has just about held its own in number of subscribers and memberships in the Society. Under ordinary circumstances there would have been a handsome gain in support. But the losses of membership were much larger than normal, so that the secretary's fine work merely succeeded in holding our ground. In order that we may continue publication at the present rate, we are appealing to our members to maintain support of the journal during the depression. If each member will do what he can to find new support among those who would find membership in the Society valuable, enough will be found to offset those whose financial condition forces them to drop membership temporarily. Extension of the library support wherever possible would also be very helpful. As several years of back volumes are available, new subscribers should be encouraged to purchase all of the back numbers which are now obtainable. Such sales increase the permanent cash assets, and by interest income from these, provide enlarged funds for the Society's work.

Stephen Hales Prize Committee.—In accordance with the provisions of the by-laws governing the STEPHEN HALES prize award, the committee in charge of the award consists of Dr. A. L. BAKKE, Iowa State College, chairman; Dr. D. R. HOAGLAND, University of California; and Dr. W. W. GARNER, U. S. Department of Agriculture.

Life Membership Committee.—President D. R. HOAGLAND has appointed on the CHARLES REID BARNES Life Membership Committee the following members: DR. W. F. LOEHWING, University of Iowa, chairman; DR. C. DEUBER, Yale University; DR. R. P. HIBBARD, Michigan State College; DR. E. S. JOHNSTON, Smithsonian Institution; and DR. R. E. GIRTON, Purdue University.

Errata.—Attention is called again to the errata list at the close of the table of contents. Insertion of these corrections in the text of your volume is recommended. The assistance of authors in compiling the list is acknowledged with thanks.

Environmental Factors in Relation to Plant Disease and Injury.—A very valuable bibliography of this field has been prepared by Dr. J. D. WILSON, and published as Technical Series Bulletin no. 9, of the Ohio Agricultural Experiment Station. The price is $1.00 per copy. It contains well toward 400 references, and is indexed in such a manner as to be exceptionally usable. There is an Environmental Factor index, following which these factors are listed in alphabetic order, with the citations (serial numbers) which consider them. The main bibliography itself is alphabetically arranged by authors. A host-disease-injury index, and a host index with scientific names in alphabetic order closes the work. The bulletin carries over 200 pages of indexed material. For sheer helpfulness it would be hard to imagine anything more useful in a bibliography. Everyone interested in environmental factors in health and disease of plants will find the bulletin indispensable to thoroughgoing work in this field. The author and the station may justly take pride in this unique contribution to the facilities for research in a very complicated field of plant physiology and pathology.

Leaf Roll Disease.—One of the Monographien zum Pflanzenschutz, no. 8, deals with the leaf roll disease of the potato. It is by Dr. F. ESMARCH, and is published by Julius Springer, Linkstrasse 23–24, Berlin W9. The monograph approaches the problem from the historical and geographic distribution standpoint, and the growing significance of the disease in agriculture. It takes up the histology and physiology of the disease, especially the effects upon carbohydrate and protein metabolism, respiration and transpiration. The methods of transfer are discussed, the influence of environmental factors upon it, the internal factors, and the etiology of the disease. The price in brochure binding is 9.9 RM. Orders may be sent directly to the publishers.

Anthokinetics.—An attractive and interesting bulletin on the physiology and ecology of floral movements has been published recently by the Carnegie Institution, of Washington, D. C., as Bulletin no. 420. The authors are Dr. G. W. GOLDSMITH and Dr. A. L. HAFENRICHTER.

The first chapter presents a resumé of the previous work on floral kinetics, the present state of knowledge concerning the mechanism of movements of floral organs, and the methods used in the observational and experimental work. To provide controllable conditions of temperature, humidity, and light, a chamber called an ecostat was developed. This control chamber is described in detail. The observations of flower movements were made under accurately controlled conditions. The species studied fall into four habit groups: heteranthous, ephemeral-heteranthous, nyctanthous, and ephemeral-nyctanthous.

Following the introduction there are eight sections dealing with particular species, which include *Tulipa gesneriana, Taraxacum officinale, Aster bigelovi, Tragopogon porrifolius, Eschscholtzia californica, Gentiana amarella, Mentzelia nuda,* and *Oenothera caespitosa,* with many others of similar habit.

The final chapter discusses the field behavior, the behavior under controlled conditions, temperature relations, light relations, humidity effects, and the mechanism of floral movements as exhibited by bracts, sepals, and petals. The work is profusely illustrated, with 34 text figures and 29 plates, 27 of which are of beautiful photographs arranged to show the cycle of floral movements of as many different species during their blooming period. These alone make the bulletin very attractive. Copies may be obtained from the Carnegie Institution of Washington, D. C., at $3.00 per copy in paper binding, and at $4.00 in cloth.

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